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OBITUARY

Geoffrey Leonard Bailey

By the death on 24 April 1955 of GEOFFREY LEONARD BAILEY, B.Sc., Ph.D., at the age of 37, dairy science has lost a worker who, with great courage and persistence in the face of a crippling physical handicap, won the unqualified respect and admiration of all who knew him, and merits a tribute in this *Journal* to which he was from time to time a contributor.

Bailey was born on 5 January 1918, in Highgate, and was educated at University College School, Hampstead. Until the outbreak of the war he was employed by Lloyds. Joining the Royal Navy, he took part in the evacuation of Narvik and saw action in the North Atlantic, where he was engaged on anti-submarine and convoy service. As a result of his war service he developed rheumatoid arthritis which led to his retirement with the rank of Lieutenant R.N.V.R. in 1944. He then entered the University of Reading, and after obtaining the degree of B.Sc. (Agriculture) in 1947 he joined the staff of the Feeding and Metabolism department of the National Institute for Research in Dairying, where he continued until the time of his death.

Bailey's career in agricultural research was for the regrettably short period of eight years, yet within this time his work in the field of animal nutrition could be equalled by few other workers. This was in spite of the fact that for most of this period he was rendered almost completely immobile by his disability, which progressively became worse. He had a keen analytical mind and a mathematical ability which revealed themselves in the many scientific papers with which his name was associated. His main scientific achievement was an original approach to animal nutrition experiments. While he used the accepted classical experimental designs, he will be particularly remembered for his application of multiple regression analysis to this type of work. In particular, he was able to make notable advances in growth studies of young dairy stock, in the study of the variations in the solids-not-fat content of milk and the rate of milk secretion. For his work he was awarded the degree of Ph.D. of the University of Reading.

By his colleagues Bailey will be remembered not only for his ability and courage, but also for his generosity to the many who came for advice and encouragement. His readiness to give help and advice, which his special aptitude particularly fitted him to render, was one of his distinguishing characteristics and one which endeared him to all his fellows.

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591. THE OUTDOOR REARING OF CALVES ON GRASS WITH SPECIAL REFERENCE TO GROWTH RATE AND GRAZING BEHAVIOUR

By J. H. B. ROY, K. W. G. SHILLAM and JUNE PALMER

National Institute for Research in Dairying, University of Reading

(With 1 Plate and 5 Text-figures)

INTRODUCTION

The standard method of rearing young dairy stock in New Zealand is the feeding of whole milk for the first 3 weeks of life and skim milk thereafter until the calves are weaned at 18 weeks of age. This takes place in a paddock close to the milking shed, the calves being then kept on rather poor pasture. This system has been generally recommended and widely adopted for a number of years. More recently, calves have been rotationally grazed on milking quality pastures in front of the dairy herd from as early as the end of the second week of life; whole milk only was fed and the calves were weaned at 8 weeks of age. Reports of trials in progress at Ruakura Animal Research Station (1,2,3,4,5) indicate that such a system will result in significantly better growth rates than for calves reared by the traditional method. Thus, over a period of 10 years, early weaned and rotationally grazed dairy calves (Jerseys) were, on average, 105 lb. heavier at 8 months of age and 158 lb. heavier at 20 months than calves reared by the standard or paddock system (3,5).

As calves of commercial dairy herds in Great Britain are normally reared indoors for at least the first 6 months of life, it was of interest to find whether a system of outdoor rearing of spring-born calves would be successful under the more severe weather conditions of this country and where the growth of grass is limited to a shorter period of the year than in New Zealand. Two trials were made with spring-born calves over two successive years, the second trial being designed in part to find out the effect of month of birth on growth rate during the first 6 months of life.

EXPERIMENTAL

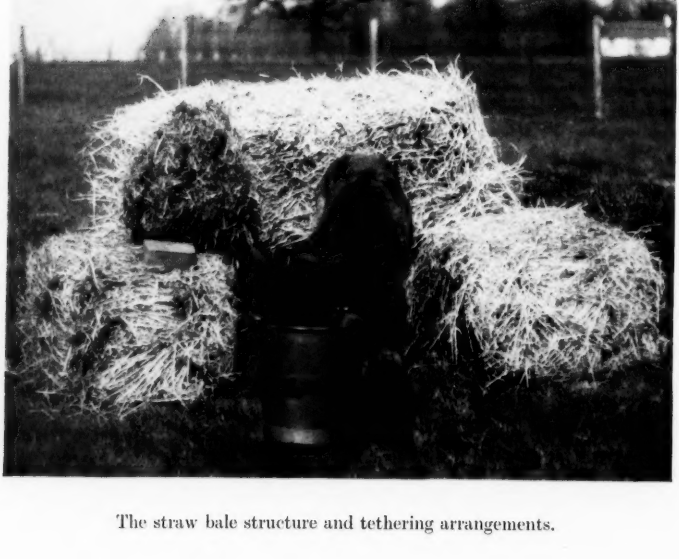
Calves

Bull calves of the Dairy Shorthorn breed were used in the experiments and were obtained from a number of commercial dairy herds in the Reading area.

Exp. 1. Five calves, three born in February and two in March 1953, were removed from their dams at birth and transported within a few hours to this Institute where they were weighed and immediately put in a grass paddock.

Exp. 2. Twelve calves, four born in each of the months of February, March and April 1954, were treated similarly.

The calves in Exp. 1 were weighed daily during the first 8 weeks of life and once weekly thereafter, whereas those in Exp. 2 were weighed at weekly intervals throughout the



The straw bale structure and tethering arrangements.

(*Facing p.* 253)

whole 6-month period. In both experiments, rectal temperature and consistency of faeces were observed during the first 3 weeks of life. Rectal temperatures above 102·8° F. were regarded as high, this being the upper limit for normal temperatures of calves in indoor experiments at this Institute during the first 3 weeks of life (6). Faeces samples were collected at weaning and at 6 months of age and examined for trichostrongyle eggs and lungworm larvae.

The calves in both experiments were tethered separately for the first 3–4 weeks in close proximity to a structure of four straw bales (Pl. 1) which were arranged so that they provided some protection from the prevailing wind and rain. The straw bales were moved to fresh ground every few days, until at castration at 1 month of age the calves were run together untethered on a restricted area of grass. At this stage several straw bales were placed in the paddock as a wind break behind which the calves could lie.

Feeding during pre-weaning period

All calves in Exp. 1 received a total of 6 pt. colostrum at 37° during the first three feeds, followed by whole milk which was fed, up to a maximum of 11 lb. daily, at the rate of 1 lb./10 lb. live weight. Three of the calves received milk at 37° for the first month of life, at which time they were changed over to cold milk which had been stored just above freezing-point, while the other two calves received cold milk from birth. Feeding was three times daily for the first 10 days of life and twice daily thereafter. If a calf scoured, the daily milk allowance was, in both experiments, reduced to a maintenance level until the faeces returned to normal consistency.

The calves in Exp. 2 received 8 pt. colostrum at 37°, followed by whole milk at the same level as in Exp. 1, feeding being twice daily throughout the entire milk-feeding period. For the morning feed the calves received warm milk direct from the cowshed, but in the afternoon the milk fed was colder (*c.* 10°), having been allowed to remain since morning collection in a churn which was placed near the calves and protected from excessive changes in temperature by straw bales.

In both experiments iodized salt licks and water were available from the first week onwards, together with dried grass of 11·8% crude protein (on a dry-matter basis) in Exp. 1 and 16·7% in Exp. 2, until the calves were 1 month of age. Very little dried grass was eaten in either experiment, the calves in general preferring pasture grass. The February, March and April-born groups of calves in Exp. 2 consumed a total of 6·5, 4·0 and 1·0 lb. dried grass respectively during that time. From the fifth week the quantity of milk fed was gradually reduced, until it was finally eliminated from the diet at 8 weeks of age. The calves were then entirely dependent on pasture grass.

The paddock used in Exp. 1 was a 2-acre permanent pasture containing about 20% white clover and ryegrass, the remainder being mainly poor productive species. Two fields were available to the calves in Exp. 2 during the pre-weaning period, one being a 3-acre permanent pasture similar in composition to that used in Exp. 1 and the other a meadow fescue-clover ley. After the first month of age, all calves were folded by means of an electric fencing unit usually with a single wire at a height of 18–24 in., a fresh allowance of grass being given daily. A back fence was erected and moved forward daily during the first few weeks in the spring of 1953, but its use was then discontinued as it was apparent that grazing was almost wholly concentrated on the fresh area.

Feeding during post-weaning period

Exp. 1. The area of pasture given each day varied with the age of the calves and with quantity and quality of the grass; about 35–40 sq.yd. of fresh grass daily were allowed for each calf at the beginning of the experiment, but this area was increased to 60 sq.yd. in July and August. As no other pasture was available, repeated folding over the same ground had to be adopted. Since the grass became relatively mature in midsummer, on 19 August supplementary feeding of 5–7 lb. artificially dried grass/calf/day (11·8% crude protein on a dry-matter basis) was begun. At this time, the mean age of the calves was just over 24 weeks. Wheat straw was available to the calves until the end of June, when, owing to the increasing fibrous nature of the grass, it was considered no longer necessary.

Table 1. *Type of pasture strip-grazed in Exp.* 2, *together with the mean live-weight gains of the calves after weaning*

No.	Type of pasture	Area (acres)	Period of grazing: From	Period of grazing: To	Time interval from previous defoliation (days)	No. of calves	Mean live-weight gain/day (lb.)
1	Permanent pasture	2·8	11 Feb. 1954	9 Apr. 1954	(Autumn 1953 to 11 Feb. 1954)	Calves not weaned	
2	Meadow fescue-timothy ley	3·5	10 Apr. 1954	5 May 1954	(Autumn 1953 to 10 Apr. 1954)	3	1·17
1	Permanent pasture	2·8	6 May 1954	9 July 1954	26	9	1·30
3	Cocksfoot ley (mature)	2·0	10 July 1954	28 July 1954	38	11	1·10
4	Cocksfoot ley	8·0	29 July 1954	13 Aug. 1954	23	11	1·69
1	Permanent pasture	2·8	14 Aug. 1954	7 Sept. 1954	35	9	1·30
5	Tall fescue-timothy ley*	9·5	8 Sept. 1954	22 Oct. 1954	55	5	2·30

* Calves had free range on this pasture.

Exp. 2. At the beginning of this experiment 30–35 sq.yd. of fresh grass/day were allowed for each calf, but thereafter the calves were, in general, given a larger area. The various pastures used in Exp. 2 are given in Table 1. Throughout the whole experiment no supplementary foods were given except that wheat straw was available at all times.

Post-experimental treatment of calves reared in Exp. 1

To find whether calves reared from birth on pasture during the spring and summer could successfully be reared for the 6-month winter period without the use of concentrated food, the calves reared in Exp. 1 were outwintered on silage from the beginning of October. A small amount of hay (3·6 lb./day) was fed for a 4-week period only. During the following spring and summer the animals were reared to 18 months of age on pasture.

Behaviour studies

In March 1953 a preliminary trial was carried out in which the movements and actions of two calves were observed and recorded between 6 a.m. and 6 p.m. Further behaviour studies of the five calves in Exp. 1 were made monthly during the following 5 months, each study being for a 24 hr. period. The detailed methods, results and discussion of these observations are reported at the end of this paper.

RESULTS

Mortality and Health

In Exp. 2, one of the calves of the March-born group (WG 2/4) died at 5 weeks of age following castration. A post-mortem examination showed that death was due to haemorrhage from the spermatic artery into the peritoneal cavity. A further calf (WG 2/2) in the same group showed lesions of calf diphtheria (*Fusiformus necrophorus*) during the sixth week of life.

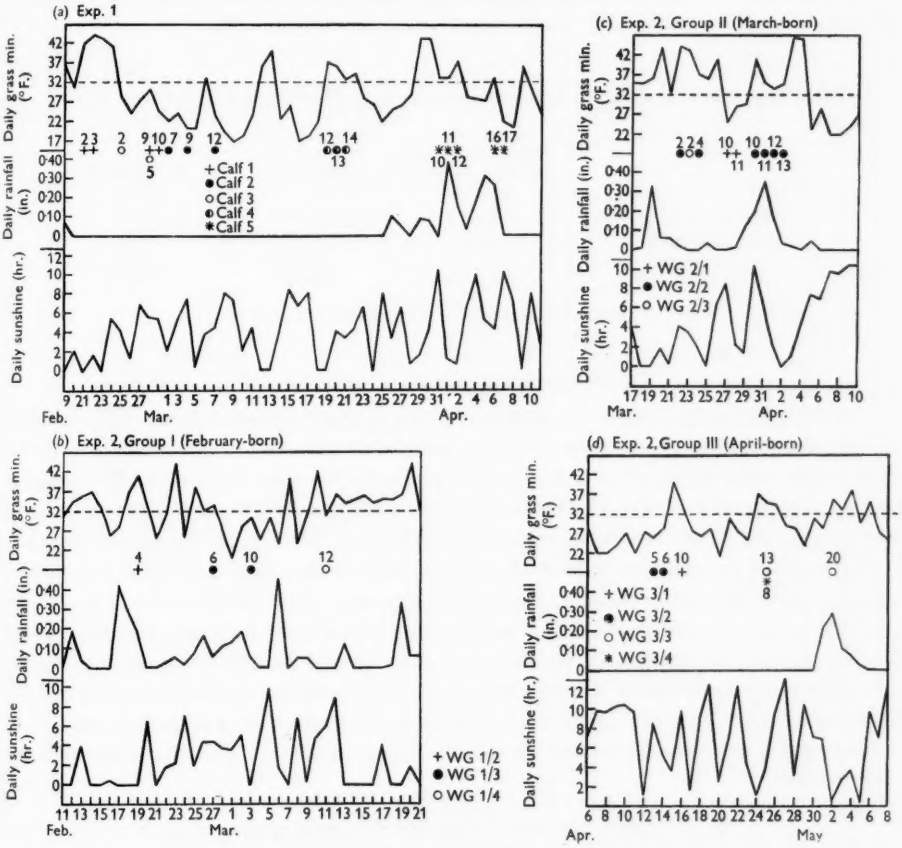


Fig. 1. Weather conditions and incidence of scouring during the first 3 weeks of life. (The symbols represent the date on which scouring occurred; the figures refer to the age of the calf in days at that time.)

This condition responded to treatment with penicillin and sulphanilamide. Another calf (WG 3/1) developed a *Corynebacterium pyogenes* abscess of the lower jaw at 12 weeks of age. The encapsulated abscess was removed a fortnight later.

The mean numbers of days on which scouring occurred are given in Table 2. In general, periods of scouring were few and did not last long. The relationship between weather conditions and the incidence of scouring during the first 3 weeks of life are shown in Text-fig. 1*a–d*. Although the rainfall during the spring of 1953 was unusually low, the

nights were rather cold, on two occasions during March the daily grass minimum temperature falling to as low as 17° F. Even so, there is no indication from Text-fig. 1 that weather conditions had any marked effect on the incidence of scouring. Although conditions were milder in Exp. 2, the rainfall during the first 3 weeks of life of the calves was greater than in the preceding year. Scouring was, on the whole, less than in 1953, and again there was no apparent correlation between weather conditions and scouring.

From Table 2 it can be seen that the mean rectal temperature of the calves during the first 3 weeks of life was normal, although in the March-born calves (Group II) in Exp. 2 the incidence of an abnormally elevated temperature was high.

Table 2. *Details of scouring and rectal temperature during the first 3 weeks of life*

	Mean no. of days on which scouring occurred*	Mean no. of days with high temperature (>102·8° F.)*	Mean temperature (° F.)
Exp. 1	3·4±0·5	1·8±0·4	101·8
Exp. 2: Group I	1·0±0·4	2·0±0·4	102·0
Group II	3·0±1·5	5·3±1·5	102·4
Group III	1·5±0·3	0·3±0·0	101·8

* Values with their standard errors of the mean.

Growth rate during the pre-weaning period

The daily live-weight gains, calculated by fitting a linear regression to the daily weights of the calves in Exp. 1 and to the weekly weights of those in Exp. 2, are given in Table 3. The weight gains for this period were possibly slightly less than those normally obtained with calves reared indoors on a similar quantity of whole milk. The rate of growth during the first month of life was, in all cases, markedly lower than during the second month.

Pre-weaning growth rates in both experiments were similar, Exp. 2 verifying the results obtained with the smaller number of calves in the previous year. In the second experiment the rate of growth during the first 8 weeks of life of the April-born group of calves was higher, though not significantly so, than that of the groups born in February and March. The regression coefficient of live-weight gain on birth weight, together with its standard error, is given in Table 3. Birth weight had a marked effect on weight gain, and after adjustment of live-weight gain for this variable, the growth rates of the three groups were very similar.

Growth rate from weaning to 6 months of age

Table 4 shows the mean live-weight gains during each month of the post-weaning period and for the whole experimental period. The calves suffered no check at weaning and a satisfactory growth rate was maintained on pasture, except in Exp. 1 from the middle of July onwards. Text-fig. 2*a* shows that after this time the weekly weight gains in this experiment decreased until supplementary feeding in mid-August restored growth rates to their previous level. If dried grass had been given in July when the increasing fibrous nature of the grass was beginning to have an effect, it is probable that heavier weights would have been reached at 6 months of age. Nevertheless, the mean live weights of 315 lb. at 6 months for the calves in Exp. 1 and 318 lb. for those in Exp. 2 are only slightly inferior to the figure of 330 lb. obtained by Bartlett & Jameson(8) with normal

Table 3. *Details of growth rates and milk consumption during the first 8 weeks of life*

Table 3. *Details of growth rates and milk consumption during the first 8 weeks of life*

Exp. 1 (1953)	Calf	Date of birth	Weight (lb.)		Live-weight gain (lb./day)			Colostrum and milk consumption (lb.)
			Birth	Weaning	Birth to weaning	0–4 weeks	4–8 weeks	
	1	19 Feb.	96·00	154·50	1·09	0·49	1·31	482·1
	2	23 Feb.	89·50	147·50	1·17	0·84	1·32	480·9
	3	23 Feb.	93·50	149·00	1·06	0·70	1·34	487·8
	4	7 Mar.	86·00	138·00	1·07	0·51	1·42	464·7
	5	21 Mar.	86·50	134·00	0·93	0·44	1·28	451·6
	Mean		90·30	144·60	1·06	0·60	1·33	473·4
Exp. 2 (1954):								
Group I (February-born)	WG1/1	11 Feb	82·00	135·00	1·03	0·56	1·29	466·1
	WG1/2	15 Feb.	80·00	124·50	0·79	0·44	0·96	460·3
	WG1/3	21 Feb.	95·00	161·50	1·26	0·86	1·51	485·4
	WG1/4	27 Feb.	84·00	137·50	1·04	0·66	1·19	479·0
	Mean		85·25	139·63	1·03	0·63	1·24	472·7
	Adjusted mean†				1·08±0·08			
Group II (March-born)	WG2/1	17 Mar.	81·50	141·75	1·04	0·64	1·49	468·7
	WG2/2	20 Mar.	74·00	103·75	0·58	0·41	0·46	450·6
	WG2/3	21 Mar.	95·75	157·75	1·23	0·84	1·49	485·4
	WG2/4	22 Mar.‡						
	Mean		83·75	134·25	0·95	0·63	1·15	468·2
	Adjusted mean†§				1·05±0·10			
Group III (April-born)	WG3/1	6 Apr.	82·00	134·50	0·99	0·46	1·39	440·3
	WG3/2	8 Apr.	94·00	155·25	1·16	0·61	1·61	486·1
	WG3/3	12 Apr.	98·00	163·25	1·21	0·83	1·47	498·3
	WG3/4	17 Apr.	97·50	168·25	1·29	0·77	1·71	491·8
	Mean		92·88	155·31	1·16	0·67	1·55	479·1
	Adjusted mean†				1·03±0·09			
Exp. 2	Mean		87·61	143·86	1·06	0·64	1·32	473·8

Regression coefficient of live-weight gain/day from birth to weaning on birth weight (Exp. 2) = 0·0239±0·0045**.

** Significant at $P<0·01$
† Adjusted for birth weight. Mean values with their standard errors.
‡ Died at 5 weeks of age.
§ Replacement value for WG2/4 calculated by the missing-plot technique of Yates(7).

Table 4*a*. *Monthly live-weight gains during the post-weaning period*

	Calf	Weight at 8 weeks (lb.)	No. of trichostrongyle eggs/g. faeces at 8 weeks	Live-weight gain (lb./day) 3rd month	4th month	5th month	6th month	Weight at 6 months (lb.)	No. of trichostrongyle eggs/g. faeces at 6 months
Exp. 1	1	154·50	<100	1·42	1·99	1·66	1·15	349·00	400
	2	147·50	1750	1·54	1·36	1·35	1·11	323·00	1750
	3	149·00	400	1·33	1·69	1·71	1·39	345·75	850
	4	138·00	<100	1·23	1·56	1·21	0·85	285·75	550
	5	134·00	<100	1·05	1·27	0·96	1·18	271·50	800
	Mean	144·60		1·31	1·57	1·38	1·14	315·00	
Exp. 2:									
Group I (February-born)	WG1/1	135·00	750	1·34	1·41	1·67	1·37	313·00	300
	WG1/2	124·50	350	1·22	1·72	1·72	1·23	276·00	350
	WG1/3	161·50	2700	1·61	1·91	1·29	1·84	373·00	1700
	WG1/4	137·50	1000	1·43	1·28	1·18	1·37	309·25	300
	Mean	139·63		1·40	1·58	1·21	1·45	317·81	
Group II (March-born)	WG2/1	141·25	350	0·89	0·97	1·00	1·17	270·00	250
	WG2/2	103·75	<100	1·23	0·80	1·14	1·55	259·00	3450
	WG2/3	157·75	100	1·63	0·91	1·77	1·33	340·00	250
	Mean	134·25		1·25	0·89	1·30	1·35	289·67	
Group III (April-born)	WG3/1	134·50	650	1·29	1·09	1·15	1·58	302·00	450
	WG3/2	155·25	350	1·16	1·26	2·17	1·39	338·00	3000
	WG3/3	163·25	550	1·01	0·85	1·73	2·47	366·50	2300
	WG3/4	168·25	550	0·76	0·89	1·23	2·50	349·00	400
	Mean	155·31		1·06	1·02	1·57	1·99	338·88	
Exp. 2	Mean	143·86		1·23	1·19	1·37	1·62	317·80	

Table 4*b*. *Growth rates from birth to* 6 *months* (*lb./day*)†

	Mean	Adjusted mean‡
Exp. 1	1·31±0·10	—
Exp. 2: Group I	1·32±0·10	1·36±0·07
Group II	1·12±0·12	1·18±0·08§
Group III	1·31±0·05	1·22±0·07
General mean	1·27	

Regression coefficient of live-weight gain/day from birth to 6 months on birth weight (Exp. 2) = 0·0171±0·0044**.

** Significant at $P<0·01$.

† Mean values with their standard errors.

‡ Adjusted for birth weight.

§ Replacement value for missing calf calculated by the missing plot technique of Yates(7).

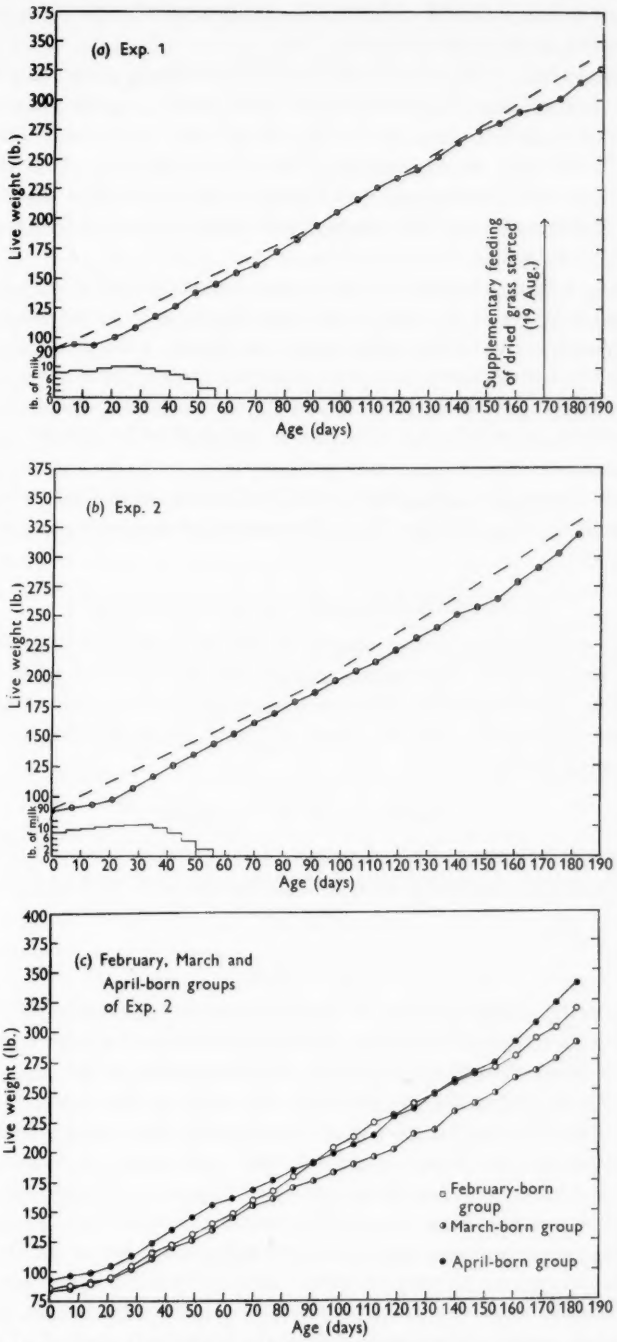


Fig. 2. Mean growth curves from birth to 6 months of age (– – –, normal growth of Dairy Shorthorn heifer calves(8)).

growth of Dairy Shorthorn heifers. This standard, together with the mean growth rate of the calves, is given in Text-fig. 2*a* and *b*.

In Exp. 1 there was a wide variation between individuals (Table 4*a*), those born in March growing less well than those born earlier in the year. As in the pre-weaning period, birth weight had a marked effect on the live-weight gain of the calves in Exp. 2; the regression coefficient with its standard error is given in Table 4*b*. After adjustment for this variable, there was an indication that February-born calves grew slightly better than those born in March and April. The mean growth rates of these three groups are shown graphically in Text-fig. 2*c*.

The mean live-weight gains/day on the various swards grazed in Exp. 2 are given in Table 1. The growth rate on leys nos. 4 and 5 was higher than on the other pastures, but on ley no. 5 the calves were allowed free range. On ley no. 2 the calves ate little but the tips of the meadow fescue and timothy leaves and did not appear to graze the clover. The cocksfoot was largely ignored on ley no. 3, and the calves restricted their attention to the clover. On leys nos. 4 and 5 grass and clover appeared to be equally grazed. On the permanent pasture it was not possible to draw any conclusions as to preference for various species. During the spring flush period the rumens of the calves became slightly distended, but at no time did they show the pot-belliedness associated with indifferent rearing.

Post-experimental growth rate of calves in Exp. 1

During the 6-month winter period the calves grew at a rate of $1{\cdot}10 \pm 0{\cdot}07$ lb./day, and during the following spring and summer the growth rate was $1{\cdot}57 \pm 0{\cdot}09$ lb./day (mean values with their standard errors). The mean live weight at 18 months of age was 796 lb., which compares favourably with the figure of 720 lb. obtained with normal growth of Dairy Shorthorn heifers (8).

Degree of parasitic infestation

No lungworm larvae were detected in any of the samples analysed. The numbers of trichostrongyle eggs/g. faeces for each calf at weaning and at 6 months are given in Table 4*a*.

DISCUSSION

Calves in commercial dairy herds in this country are normally reared indoors on concentrates and hay following a milk-feeding period the duration of which varies from farm to farm and are seldom allowed to graze until about 6 months of age and sometimes very much later. One of the reasons for following this policy is that grass, when comprising a major part of the diet, has for some time been considered an unsuitable food for normal growth of the young calf. However, growth rates approximating to normal have been obtained in these experiments on an all-grass diet following a limited quantity of milk, and from the live-weight gains on the various swards grazed in Exp. 2 it seems likely that the performance of the calves would have been enhanced if better quality pastures had been available throughout the experiments. Rearing by this method has not produced spectacular increases in growth rate as in the New Zealand experiments, but it should be remembered that growth rates obtained with the traditional method of rearing in that country are poor, since, after weaning, the calves are left largely to fend for themselves in

paddocks which, owing to the calf's selective grazing habit, probably soon consist of a series of patches of under- and over-grazed grass. In the words of McMeekan (5), 'they are turned into a relatively large field containing plenty of pasture and left there for weeks and sometimes months at a time, being shifted only when it is obvious that the food supply is becoming scarce. At this stage a shift is made to a similar field and again the animals remain there for a considerable period.' By using leys at their optimum stage of growth, weight gains in the order of 2 lb./day could probably be achieved during at least the period 3–6 months of age, and possibly at an even earlier age. The method might then be suitable not only for rearing dairy-herd replacements but also for the rearing of calves intended primarily for meat production. In order to obtain maximum growth rate, the results given in Table 1 indicate that calves, probably owing to their highly selective grazing behaviour, should be allowed free range rather than be 'strip-grazed'.

Although, in Exp. 1, the calves born in March grew less well than those born in February, there was no evidence from Exp. 2 that month of birth had any significant effect on growth rate.

Previous experiments on the use of grass for the young calf have been carried out in the more equable climate of New Zealand, and it was therefore assumed that it would not be so easy to rear young calves under British climatic conditions with the wide variation in air temperature between day and night during the spring months. Our experiments indicate, however, that new-born calves, of the Dairy Shorthorn breed at least, possess a higher degree of hardiness than is generally supposed. Cold weather seemed to have little effect on the well-being of the calves as judged by scouring, and this has been further demonstrated in experiments at this Institute with calves placed out of doors at birth during the autumn and early winter months (9). In America, calves reared from birth or a few days of age in portable pens or open shed-type houses have been found to exhibit a marked lowered incidence of scouring, respiratory troubles and mortality than calves reared in conventional barns (10, 11, 12). Similar findings have been reported from Russia with calves and lambs (13). No adverse effects have been observed with temperatures as low as 9° F. (11) or −4° to +4° C. (13), although climatic conditions were assumed to be responsible for the slightly reduced growth rates of outdoor-reared calves during the first 2 weeks of life (12) and during the first 8 weeks (14). The somewhat low live-weight gains during the first month of life of the calves in our experiments are probably likewise a result of low environmental temperatures, but it is not known whether calves kept indoors during say the first 2 or 3 weeks of life before being put out to pasture could be weaned so easily on to grass at an early age and whether a sudden downward change in environmental temperature, other than at birth, would be hazardous.

The high susceptibility of young stock to parasitic infestation is generally purported to be a further factor against rearing on pasture from an early age, and in our experiments the trichostrongyle egg-counts were, in many cases, well above the values generally recognized as indicative of a pathogenic infestation in older animals (15). The calves were not treated with an anthelmintic, however, as the heavy burden did not seem to depress growth unduly, and, in fact, some of the best growth rates were obtained with calves showing the highest egg-counts. It is possible that the exposure of very young calves to worm infestation enables the building up of a resistance, as occurs in older stock, at an earlier age than in calves reared by more conventional methods. From the results of our experiments, which were carried out on pastures not previously stocked with cattle for

about 6–8 months, there seems little risk of calves developing symptoms of parasitic gastro-enteritis provided a plentiful supply of young grass is always available.

It is possible that calves reared on grass from birth develop larger rumens than those reared by more conventional methods, thus enabling the mature animal to deal more effectively with large amounts of bulky food. From the post-experimental results in Exp. 1 it is clear that the calves made good progress when reared on silage and grass alone to 18 months.

An analysis of quarterly returns by Phillips(16) in 1950 showed that approximately 27% of total calf births in England and Wales occurred during the months February–April. If a large proportion of the calves of dairy breeds born during this time could be successfully reared out of doors on grass to about 6 months of age, the saving in cost would be considerable.

BEHAVIOUR STUDIES

Methods

Observations by a team of three workers operating on a shift system began at 4.30 or 5.30 a.m. and, except for the preliminary observation in March, continued for 24 hr. The movements and actions of the calves, except for defaecations, urinations, drinks of water and visits to the salt lick, were recorded at 2 min. intervals. The record made was the specific action of the calf at the time of recording and not necessarily during the preceding 2 min., the chance being that any errors so introduced would cancel each other out over a period of 24 hr.

During the hours of darkness it was relatively easy on a moonlight night to observe and record the actions of the calves with the naked eye. On one occasion when the sky was cloudy the portable infra-red ray equipment of Burton & Castle(17) was employed, but was found to have limitations in range and in the time taken to adjust the eye to the equipment. The limited use of a torch on a further cloudy occasion did not seem to disturb the calves in any way.

Definitions

Behaviour categories were defined as follows:

Grazing time. The total time spent eating herbage, both while standing (including short periods of walking while selecting grass for eating) and lying.

Rumination time. The total period of time spent in regurgitation, mastication and deglutition of ruminal ingesta, including the short time intervals between boluses.

'*Hygiene*' *time.* The time spent during which actions were of a cleansing or anti-irritant nature.

Frisking time. The total time during which actions were of a playful nature.

Walking time. The time spent moving from one area to another with the exception of short periods of movement whilst grazing.

Standing time. The time spent in an erect stationary position, but excluding grazing time.

Lying time. The entire time spent with the body resting on the ground.

Results and discussion

Some details of weather conditions during the 24 hr. periods are given in Table 5 and the results of the observations in Table 6. The results for the preliminary trial in March 1953 are included in Table 6, but the values were not used in calculating the general means.

Table 5. *Details of weather conditions during the observations*

Date	Rain (in.)	Temperature (° F.) Max.	Min.	Grass min.	Sunshine (hr.)
17–18 Mar. 1953	—	58·0	26·2	18·2	8·0
22–23 Apr. 1953	—	66·0	38·8	31·5	12·6
20–21 May 1953	0·03	67·0	42·0	34·0	10·1
25–26 June 1953	—	79·0	46·0	40·0	7·9
28–29 July 1953	0·01	75·6	46·5	41·0	8·4
25–26 Aug. 1953	—	69·0	55·0	51·0	7·6

Table 6. *Data from 24 hr. observations made in 1953 on five Shorthorn calves*

Date of observation ...	17–18 Mar.*	22–23 Apr.	20–21 May	25–26 June	28–29 July	25–26 Aug.	General mean†
Mean age of calves (days)	22·0	51·2	79·2	115·2	148·2	176·2	114·0
Mean time spent (hr.):							
Grazing	1·30	5·59	7·59	8·87	9·34	4·87	7·85‡
Ruminating	1·23	6·83	6·95	7·75	8·89	9·10	7·61‡
'Hygiene' time	1·19	1·43	1·27	0·60	0·59	0·47	0·87
Frisking	0·13	0·05	0·05	0·07	0·03	0·04	0·05
Walking	0·57	0·53	0·35	0·22	0·30	0·29	0·34
Standing	3·74	3·65	4·03	2·60	1·61	4·89	3·36
Lying	6·23	14·26	12·06	11·69	12·73	13·95	12·94
Eating dried grass	0·88	0·18	—	—	—	2·87	—
Eating straw	0·23	0·27	0·45	0·36	—	—	0·36
Mean number of:							
Defaecations	1·5	5·8	7·0	12·2	9·4	13·2	9·5
Urinations	6·0	12·6	20·0	18·6	13·4	11·8	15·3
Drinks of water	3·5	5·2	15·8	12·6	6·4	5·4	9·1
Visits to salt lick	1·0	0·0	0·8	5·4	2·0	2·0	2·0

* Two calves only, observed from 6 a.m. to 6 p.m.
† Excluding observation on 17–18 Mar.
‡ Excluding observation on 25–26 Aug. (see text).

GRAZING BEHAVIOUR

Grazing time. In both experiments from about the 7th day onwards much time was spent by the calves attempting to graze, and although one calf did not succeed in actually biting off grass until it was over 3 weeks old, in general, the art of grazing was mastered well before that time. The colour of the faeces was recorded daily; some green colouring matter was usually present at the 7th day, whereas by the 14th–16th day the faeces had turned the dark green colour normally exhibited by the faeces of mature cattle at grass.

The relationship between length of grazing time and age of calf was found to fit the following regression equation;

$$Y = 7{\cdot}25 + 0{\cdot}161\ (x_1 - 91{\cdot}5) - 0{\cdot}0006\ (x_2 - 10183{\cdot}1),\ \text{S.E.} = \pm 0{\cdot}81,$$

where Y = grazing time (hr.), x_1 = age (days) and x_2 = age^2 (days). This regression line and the actual times spent grazing are given in Text-fig. 3. Observations made in August are not included, since dried grass was being fed at that time.

Hancock has shown, both from extensive grazing studies of mature cattle (18) and from his review of the literature (19), that grazing time is likely to be high when abundant amounts of herbage of varying fibre content are offered. In view of this, it is probable that the high values for grazing time given in Table 6 were at least partly due to the somewhat poor quality of the pasture. Thus McArthur (20), from a summary of fortnightly 24 hr. observations of calves from 3 to 9 months of age, found the average grazing time during this period to be 7·57 hr./day on milking quality pasture and 9·28 hr. for a second group on poor-quality pasture. However, with seven pairs of identical twins, Corbett (21) observed that the duration of high and low intensity grazing on good-quality ryegrass and clover pasture was as great as 7 hr. in a 24 hr. period at 11 weeks of age.

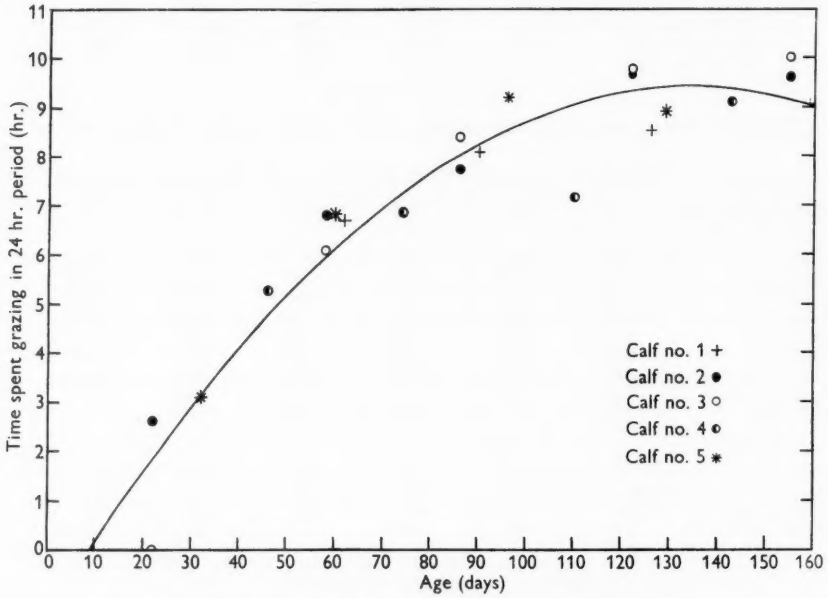


Fig. 3. The relationship between age and the time spent grazing in a 24 hr. period.

Time spent grazing in a lying position. When the calves were very young, an appreciable portion of the total grazing time took place while they were lying, but this decreased rapidly with age. During the first observation, out of 1·30 hr. total grazing from 6 a.m. to 6 p.m., 0·32 hr. or 24·6% took place in the lying position. During the second and third observations the corresponding figures were 2·0 and 1·2% and during the fourth and fifth 0·6 and 0·2%. When grazing took place in this position, it occurred immediately on lying down, and once the calves had settled, little or no grazing took place.

Distribution of grazing time. As the daily pattern of grazing behaviour in each monthly observation was remarkably similar, the mean time spent grazing in each ½ hr. expressed as a percentage of the total time was calculated for each of these observations. A histogram of these percentages, averaged from all five monthly observations and corrected to Greenwich mean time where necessary, is given in Text-fig. 4*a*. Very little grazing took place during the hours or darkness, but throughout the day there were definite peaks of

grazing intensity, the first beginning at approximately 5 a.m., irrespective of time of sunrise. For instance, in June grazing began at an average time of 67 min. after sunrise, whereas during the August observation when the time of sunrise was 77 min. later, grazing began 10 min. before sunrise. The relationship between time of first grazing period and time of sunrise is shown in Text-fig. 5. The second grazing peak occurred when the electric

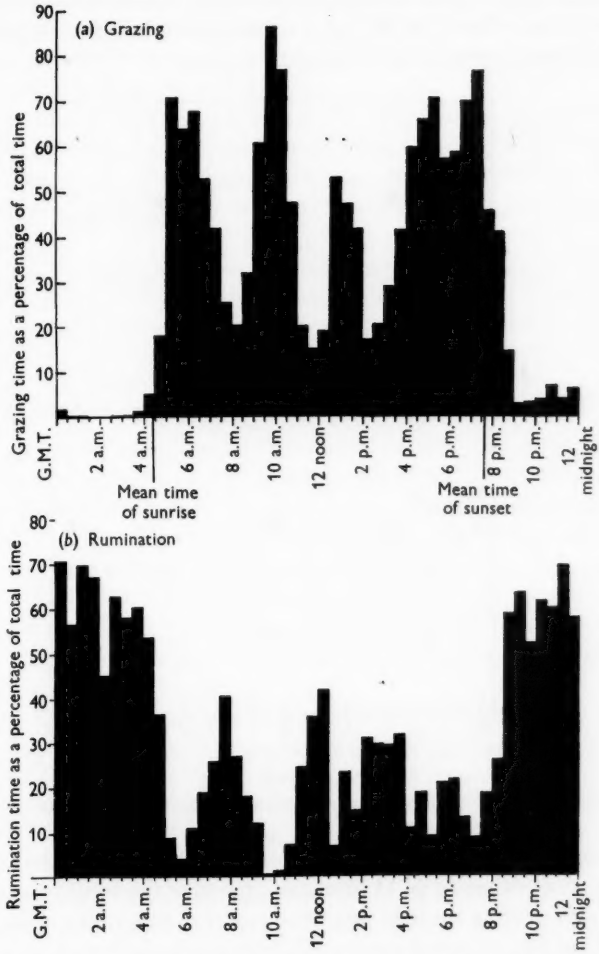


Fig. 4. The distribution of grazing and rumination throughout the day (mean of five monthly observations).

fence was moved at approximately 9 a.m. and the third, which was less intense and shorter in duration, during the early afternoon. The final period of grazing, which began in the late afternoon and continued until 45–70 min. after sunset (Text-fig. 5), was more prolonged than any of the previous three periods. With bullocks and sheep, Hughes & Reid (22) similarly found the cessation of evening grazing to be correlated with sunset, but in

contrast to our observations with calves, they also found commencement of early morning grazing from May to September to be correlated with sunrise.

This periodicity of grazing of young calves is, with one exception, very similar to that observed with bullocks by Tayler (23); whereas a first period of grazing beginning near sunrise existed for a group of eight bullocks in a 2-acre paddock, it was absent for a second group which were 'strip-grazed', the fence being moved daily at approximately 9 a.m. Similar findings have been reported with dairy cattle (24). During the course of our observations, there was no indication that the calves withheld their first grazing period until later in the morning in anticipation of a fresh supply of grass.

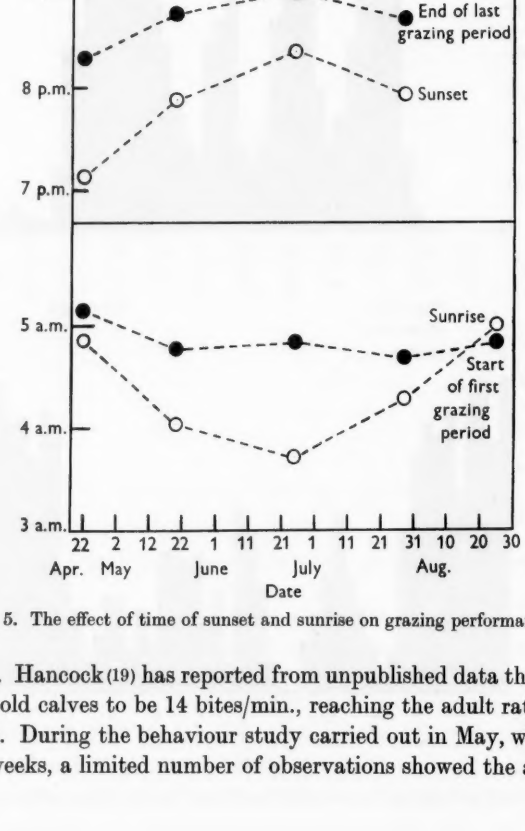


Fig. 5. The effect of time of sunset and sunrise on grazing performance.

Speed of grazing. Hancock (19) has reported from unpublished data the average speed of grazing of 8-week-old calves to be 14 bites/min., reaching the adult rate of 50 bites/min. at 18 weeks of age. During the behaviour study carried out in May, when the mean age was just over 11 weeks, a limited number of observations showed the average rate to be 28 bites/min.

RUMINATION BEHAVIOUR

Rumination time. The mean times spent ruminating during the observations (Table 6) steadily increased with age of the calf. At a mean age of just over 7 weeks, almost 7 hr./day were spent in rumination. A similar figure was obtained by Johnstone Wallace & Kennedy (25) for mature beef animals and a somewhat lower figure (5·60 hr.) by Castle,

Foot & Halley (26) for lactating dairy stock. The long ruminating times in our experiments probably resulted from the poor quality of the pasture, for it is well known (18,19) that time spent ruminating tends to be lower on high-quality pasture. On the other hand, McArthur (20), in a summary of fortnightly observations on calves from 3 to 9 months of age, found little or no difference in rumination time between groups on good- and poor-quality pasture, the times spent ruminating being 6·92 and 6·88 hr. respectively.

Probably because of the favourable weather conditions, the calves preferred to ruminate lying down, only 5·2% of the total rumination time being spent in the standing position. The start of rumination when standing was usually an indication that the calf would shortly lie down and continue ruminating.

Distribution of rumination time. To illustrate the periodicity of rumination throughout the day, Text-fig. 4*b* shows the time spent ruminating in each ½ hr. period calculated as for grazing in Text-fig. 4*a*. Rumination took place mainly during the hours between sunset and sunrise with smaller intermittent peaks in the daytime during intervals between grazing.

Rumination rate. Rates of rumination have been quoted by Hancock (19) of 82 bites/min. at 6 weeks of age dropping to 67 bites/min. at 9 months. During the May observation, when the mean age was just over 11 weeks, the mean rate of rumination was 85 bites/min. and the time taken to chew one bolus was 0·6 min.

'HYGIENE' TIME. 'Hygiene' time was comprised of the time spent licking the body with the tongue, scratching with the hind leg and rubbing against posts and other objects. Table 6 shows that these activities occupied a considerable amount of time when the calves were young and decreased as they became older. From our observations it appears that these activities are most likely to take place early in the mornings at the beginning of grazing and in the evening on settling down for the night. At no time during the period under investigation were external parasites observed on the calves.

FRISKING TIME. The mean time spent frisking during the five observations was 3·0 min. Frisking took place during the hours of daylight only and was most common following movement of the electric fence and during the period after sunset between the end of grazing and settling down for the night.

WALKING TIME. The mean time spent walking was 0·34 hr. Walking time was comprised of breaks in grazing while walking from one area to another, visits to water and salt lick, searching for suitable areas to lie down and apparently aimless wanderings.

STANDING TIME. The average time spent standing was 3·36 hr., of which 1·37 hr. was spent more or less motionless. During the first three 24 hr. observations, a mean time of 0·36 hr. was spent eating straw while standing.

LYING TIME. The mean lying time was 12·94 hr., of which 48·2% was spent with the right flank in contact with the ground and 51·8% with the left. Brownlee (27) has observed that during long resting periods, whereas cattle frequently stand up and lie down again immediately or after a few minutes with the opposite flank in contact with the ground, this behaviour is less uniform with calves. During our observations however, of 148 breaks of not more than 6 min. in duration during long resting periods, the calves settled down with the opposite flank in contact with the ground on 113 occasions and with the same flank on only 35 occasions. Often the need to pass urine or faeces would seem to be the cause of the calf rising to its feet, but very frequently a calf was observed to stand up only to lie down again almost instantly with the opposite flank in contact with the ground.

'Sleeping' with the head on the upper flank and with eyes at least partly closed took place both during the hours of darkness and during the daytime, periods of ½ hr. spent in this position being quite common. During all 24 hr. observations, a mean of 3·19 hr. was spent in this position. Lying prostrate with the head on the ground and legs extended occupied 0·54 hr. and was far more likely to occur during the hours of daylight and especially when sunny, than when it was dark. Although Brownlee (27) has noticed that, probably due to interference with rumen functions, neither of these positions is maintained for more than about 10 min. at a time in adult cattle, during the course of our observations with calves, periods of up to 0·90 hr. were spent with the head turned round to the upper flank, and up to 2·13 hr. lying with legs and head extended.

Metabolic and other functions. Dung was voided an average of 9·5 times/day and urine 15·3 times. An average of 9·1 drinks of water/day were taken and 2·0 visits to the salt lick were made.

SUMMARY

1. Five Dairy Shorthorn male calves born in February and March 1953 and three groups of four born in each of the months February, March and April 1954 were placed out of doors on permanent pasture at birth.

2. Each calf received 6 or 8 pt. colostrum followed by approximately 45 gal. whole milk during the first 8 weeks of life, and thereafter, except for a small amount of dried grass given in Exp. 1, pasture grass comprised the sole item of diet until 6 months of age.

3. Mean growth rates for each month of life and from birth to 6 months are presented. After the first month of life, good progress was made, and it is considered that the live weights at 6 months of age are similar to those obtained with conventional methods of rearing.

4. There was a significant effect of birth weight on live-weight gain. After adjustment for birth weight, there were no significant differences in live-weight gain between February, March and April-born groups of calves.

5. Weather conditions seemed to have little effect on the incidence of scouring during the first 3 weeks of life.

6. On leys, greater weight gains were obtained than on permanent pasture.

7. No lungworm larvae were detected in faeces samples examined. In many cases there was a high level of infestation with stomach and intestinal worms as indicated by the number of trichostrongyle eggs/g. faeces. Some aspects of this infestation are discussed.

8. Satisfactory growth to 18 months of age was obtained with silage during the 6-month winter period and at grass during the following spring and summer.

9. Details of behaviour of the five calves in Exp. 1 studied at approximately monthly intervals to 6 months of age are presented.

We wish to acknowledge the interest and advice of Dr S. Bartlett. We are also indebted to Mr P. L. Ingram, Royal Veterinary College, for carrying out the faeces analyses and post-mortem examination, Mr A. S. Foot and Mr D. F. Terrill for providing grazing in Exp. 2 and Mr J. R. Lloyd, University of Reading Horticultural Station, for allowing us access to meteorological data.

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612.664.33:636.2.084

592. STUDIES OF THE SECRETION OF MILK OF LOW FAT CONTENT BY COWS ON DIETS LOW IN HAY AND HIGH IN CONCENTRATES

VI. THE EFFECT ON THE PHYSICAL AND BIOCHEMICAL PROCESSES OF THE RETICULO-RUMEN

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(With 2 Figures)

Earlier experiments at this Institute confirmed that marked falls in milk-fat content can be caused by diets which provide less than about 8 lb. hay daily and supply the remainder of the nutritional requirements of milking cows as concentrates (1,2,3). Digestibility trials suggested that a shortage of 'fibrousness' would not cause falls in milk-fat content unless the diet contained large amounts of digestible starch (4), and a later experiment (5) demonstrated that the depression is greater with flaked maize than with maize meal. The object of the present experiment was to observe the physical and chemical conditions in the reticulo-rumen of cows receiving normal diets and diets which depress the fat content of milk.

METHOD

The experiment, which lasted for 27 weeks, was carried out with two Shorthorn cows each fitted with a large rumen fistula. Cow A (Dorrit 15) calved 32 days before and was fistulated 1 week after the start of the experiment. Cow B (Halora) calved 17 days before the start and had been fistulated for over 2 years. The experiment was divided into five periods, in four of which there were periods for the collection of detailed data concerning conditions in the reticulo-rumen and the digestibility of food.

Period I (preliminary control), weeks 1–7. Each cow received 16 lb. hay and 20 lb. concentrates daily. Weeks 6 and 7 formed a collection period.

Period II (low hay), weeks 8–13. In weeks 8 and 9 the daily hay ration was reduced to 2 lb. and the concentrates increased to 24 lb. This diet was given until the end of the period. Weeks 11 and 12 formed the collection period.

Period III (low hay and straw pulp), weeks 14–19. The rationing of both cows was as in period II with the daily addition of 5 lb. dried straw pulp. In week 15 the concentrates were reduced to 20 lb. daily. Weeks 17 and 18 formed the collection period.

Period IV (low hay), weeks 20 and 21. Straw pulp was removed from the diet, the daily ration being 2 lb. hay and 20 lb. concentrates.

Period V (final control), weeks 22–27. In week 22 the daily diet was gradually changed to 18 lb. hay and 10 lb. concentrates. This diet was given until the end of the experiment. A modified collection period, involving no digestibility trial, occupied week 24.

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Foods. The hay was good-quality lucerne as used in our earlier experiment (2), and the concentrate mixture again consisted of flaked maize 10 parts, weatings 7 parts, decorticated ground-nut cake 3 parts, with 0·5% mineral mixture added. The cows had access to mineral licks at all times. The straw pulp was macerated delignified straw, prepared by treatment of straw under pressure with sodium hydroxide.

Management. The cows were housed in special standings, and during collection periods they wore apparatus for the separate collection of faeces and urine (6). When not wearing the apparatus they were allowed exercise in a concrete yard for a few hours daily. The cows were fed at 6 a.m. and 6 p.m., receiving half the daily ration at each feed except that when the diet contained only small amounts of hay these were given at the evening feed.

General experimental records. The daily intakes of food and water were recorded. Milk fat and total solids contents were determined daily on composite p.m./a.m. samples for each cow.

Collection periods

(1) *Digestibility trial and nitrogen balance.* Digestibility trials were carried out as described previously (4), except that samples of food and faeces were bulked separately for each of the 2 weeks. For nitrogen determinations composite samples of wet faeces were stored under sulphuric acid, and both composite urine and milk samples were stored at 0° C., formaldehyde being added to the milk.

(2) *The extent of digestion in the rumen.* During the second week of each collection period samples of digesta were taken from the region of the reticulo-omasal orifice for the determination by the lignin-ratio technique of the extent of digestion in the rumen. Three samples, at 4 hr. intervals, were taken per day, the time of the first sample being chosen at random between 6 and 9 a.m. The samples were bulked for each cow for analysis, subsamples being again stored under sulphuric acid for nitrogen determinations.

(3) *Physical processes of the reticulo-rumen.* (*a*) The rate of passage of hay through the gut was determined with stained hay (7). (*b*) The dry-matter content of the digesta in the dorsal and ventral sacs of the rumen and in the reticulum was determined at 12 noon on each day of the second week. (*c*) The total weight and dry-matter content of the digesta in the reticulo-rumen were measured by complete removal, weighing and sampling at 12 noon on the last day of each collection period. The contents were immediately returned. (*d*) In the first week of each collection period the contractions of the reticulum were recorded continuously. In the second and third collection periods less frequent records of jaw movements were also made. The recordings were made by means of lightly inflated balloons connected to tambours operating pens. Details of the recording apparatus and of the interpretation of such records have been published (8, 9); in this instance the balloons were held in the reticulum by means of weights and against the jaw by means of a leather head-stall. A special balloon-holder prevented the balloons in the reticulo-rumen from becoming folded.

(4) *Biochemical processes of the reticulo-rumen.* Samples of rumen liquor were taken at hourly intervals over one period of 24 hr. in the second week of each collection period for the estimation of pH, lactic and total volatile acids, and at 12 noon on this and three other days for the estimation of the proportions of the volatile fatty acids. Experiment showed that the concentration of volatile acid throughout the rumen liquor was constant and a standard method of sampling was adopted as follows. Digesta were taken from

about 6 in. below the surface of the rumen contents and the liquor strained through surgical gauze.

(5) *Composition of the milk fat.* Butter was made once each week from the milk of each cow taken at two or four consecutive milkings.

(6) *Concentration of certain blood constituents.* At 12 noon on four alternate days in the second week of each collection period samples of blood were taken from the jugular vein of each cow. The first sample, for blood glucose, was taken into an oxalated tube immediately after approaching the cow. The second sample was allowed to clot prior to the separation of the serum.

Analytical procedures

(1) *Food, faeces and rumen digesta* were analysed for their proximate constituents, and the carbohydrates were further separated into cellulose, hexosan cellulose, cellulosans, pentosans-not-in-cellulose, and starch as described previously (4). Lignin was estimated by the method of Armitage, Ashworth & Ferguson (10).

(2) Rumen liquor. (*a*) The pH was determined with a glass electrode after the samples had been cooled rapidly to room temperature. (*b*) *Total volatile acid.* After centrifuging lightly to remove suspended solids, rumen liquor was diluted with four volumes of distilled water. Equal volumes of diluted liquor and saturated acidified magnesium sulphate solution were mixed (11) and left overnight to precipitate protein. The precipitated protein was removed by centrifuging and an aliquot of the supernatant liquid distilled in the Markham still, the distillate being titrated under carbon dioxide-free conditions using phenol red as the indicator. (*c*) *Individual volatile acids.* The double distillation method of Friedmann (12) was used except that protein was removed as above before steam distillation. The mixture of fatty acids was dissolved in chloroform containing 5% v/v *n*-butanol (11) and analysed by the partition chromatographic method of Moyle, Baldwin & Scarisbrick (13) using column I. This gave a separation into acetic, propionic and butyric acids, together with a mixed fraction containing the higher homologues. (*d*) *Lactic acid.* After the removal of soluble carbohydrates and protein by precipitation with copper-lime (14), lactic acid was oxidized to acetaldehyde which was estimated colorimetrically with *p*-hydroxydiphenyl (15). The recovery of lactic acid, added (as the lithium salt) in amounts from 4 to 200 mg./100 ml. rumen liquor, varied from 98 to 103%.

(3) *Milk and milk fat.* The fat content of the milk was determined by the Gerber method, total solids by drying, and the Reichert, Kirschner, Polenske and iodine values of the milk fat were determined by the British Standard Methods (16).

(4) *Blood constituents.* (*a*) *Glucose.* The method of Nelson (17) was used after protein had been precipitated with the zinc sulphate-barium hydroxide technique. (*b*) *Blood lipids.* Total and free cholesterol were estimated in serum by the methods of Sperry & Webb (18). For each cow the residues of the four samples were bulked to give two samples for each week. Blood lipids, apart from cholesterol, were determined by the methods of Saarinen & Shaw (19).

RESULTS

Milk yield and composition. Weekly mean values for the yield of milk, its fat and solids-not-fat contents and the yield of milk fat for each cow are shown in Fig. 1. The decline in milk-fat content on the introduction of the low roughage diet in period II continued for 4 weeks after the dietary change had been completed. Once the low point

had been reached, the mean weekly value remained below 2·1% fat until the hay was restored in period V. Addition of dried straw pulp to the low roughage diet (period III) brought no recovery of the milk-fat content. From a consideration of the milk-fat yield during the initial and final control periods, the loss of milk fat due to changes in feeding was calculated to be 62·2 and 51·2% in cows A and B respectively in the periods of low

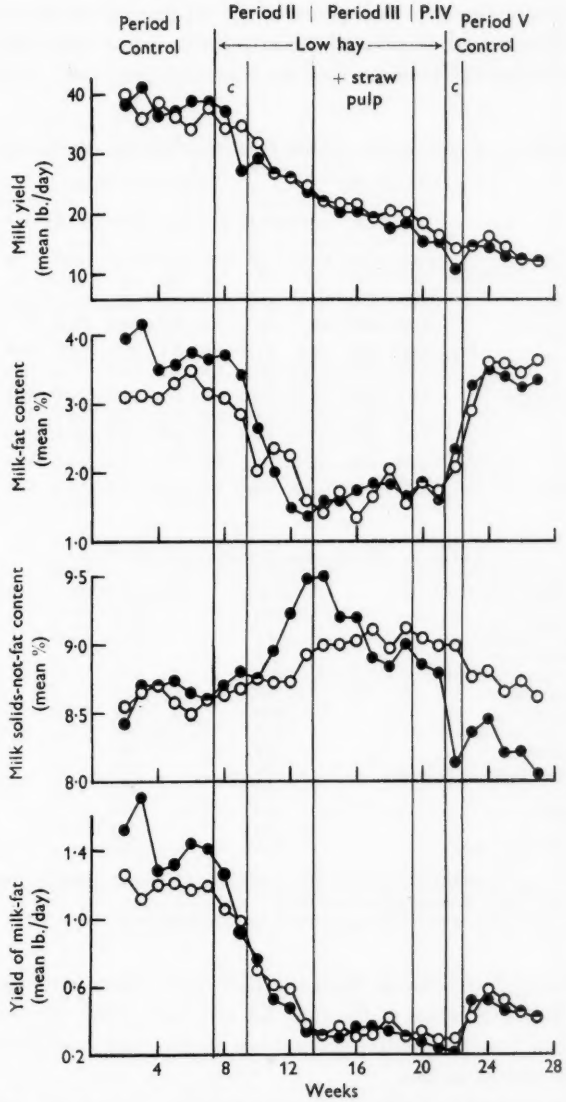

Fig. 1. The mean daily milk yield, fat content, solids-not-fat content and fat yield from cows A, ●, and B, ○. During periods I and V the cows received the control diet; during periods II, III and IV they received diets of low hay and concentrates; in period III the diets low in hay were supplemented with dried straw pulp. Weeks in which gradual changes in diet took place are marked (*c*).

hay feeding (weeks 12, 13, 20 and 21) and 62·5 and 58·7% when straw pulp was added (weeks 14 to 19).

While the milk-fat content was depressed there was an increase in the solids-not-fat percentage of the milk of both cows. Values up to 0·75 and 0·4% solids-not-fat higher than any recorded during either control period were observed with cows A and B respectively.

Composition of milk fat. The Reichert, Polenske and Kirschner values and the iodine value of the milk fat produced in each period of the experiment are given in Table 1. The decrease in the volatile acid values and the increase in iodine value are similar to, but more pronounced than, the changes that we observed previously when milk fat was depressed (1).

Table 1. *Composition of milk fat throughout the experiment*

Period of experiment	Diet	Week	Milk fat (%) on day of sampling		Volatile acid values: Reichert (*R*)		Polenske		Kirschner (*K*)		*K*/*R* × 100		Iodine value	
			Cow A	Cow B	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B
I	Initial control	6	3·62	3·38	35·0	33·4	2·4	4·0	29·4	28·1	83·9	84·2	36·9	33·8
		7	4·22	3·90	33·6	29·1	2·7	3·2	28·5	24·0	84·8	82·4	38·3	37·1
II	Low hay	11	2·00	3·00	24·8	25·0	4·0	3·1	20·8	21·0	84·0	83·9	42·9	41·8
		12	1·75	2·55	13·9	21·7	1·5	3·9	11·0	17·1	79·0	79·0	55·5	46·3
III	Low hay + straw pulp	16	1·81	1·61	13·8	10·7	1·2	1·2	11·3	8·7	82·1	81·3	53·7	55·2
		17	2·06	1·60	19·6	10·8	1·3	1·6	15·5	8·7	79·2	80·6	52·6	55·4
		18	1·92	2·38	17·9	13·1	1·3	1·7	14·7	10·4	82·2	78·8	51·6	49·4
IV	Low hay	21	1·63	1·44	16·5	10·4	1·3	1·3	13·6	8·5	82·6	82·2	54·7	59·2
V	Final control	24	3·46	3·85	25·9	18·8	1·5	1·2	20·8	14·9	80·2	79·6	39·4	45·7

Table 2. *Mean composition of foods* (%)

Food constituent	Hay	Concentrates	Straw pulp
Dry matter	83·7	85·6	95·9
Crude protein	12·1	16·3	2·2
Ether extract	1·4	3·1	0·0
Nitrogen-free extract	32·8	58·4	23·5
Crude fibre	29·8	4·5	66·3
Cellulose	29·9	7·9	77·2
Hexosan cellulose	22·6	4·8	63·3
Cellulosans	7·3	3·1	13·9
Pentosans-not-in-cellulose	7·9	7·9	4·4
Starch	0·0	29·2	0·0
Lignin	6·4	1·6	1·7
Ash	7·6	3·3	3·9
Starch equivalent	28·1*	67·0*	21·2†

* As determined from digestible nutrients in the initial control period (period I), using a value for the digestibility of hay previously determined on four cows.

† Assuming the digestibility of hay and concentrates was the same as in period I.

No marked changes were found when the Kirschner values were expressed as a percentage of the Reichert values of the milk fat secreted during the feeding of different diets. This suggests that the relative proportions of the lower volatile acids did not vary appreciably with change in diet.

Food intake and digestion. Values for the composition of the foods, the weights of each nutrient consumed and digested, and the digestibility coefficients are given in Tables 2, 3 and 4 respectively. The changes in digestibility caused by decreasing the ratio of hay to concentrates in the diet (period II) were similar to those we reported previously (4).

Table 3. *Foods and food constituents consumed and digested and water drunk: (mean lb./day) in 14-day collection periods*

Food and food constituents	Consumed						Digested					
	Period I (initial control)		Period II (low hay)		Period III (low hay + straw pulp)		Period I (initial control)		Period II (low hay)		Period III (low hay + straw pulp)	
	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B
*Hay	16·0	16·0	2·0	2·0	2·0	2·0	—	—	—	—	—	—
*Concentrates	20·0	20·0	24·0	24·0	20·0	20·0	—	—	—	—	—	—
*Straw pulp	—	—	—	—	5·0	5·0	—	—	—	—	—	—
Dry matter	28·7	30·1	22·2	20·8	23·8	23·1	20·8	21·2	17·0	15·7	17·3	16·8
Crude protein	5·0	5·1	4·2	3·9	3·5	3·3	3·6	3·7	3·3	2·9	2·5	2·3
Ether extract	0·8	0·8	0·8	0·8	0·6	0·6	0·5	0·5	0·5	0·4	0·4	0·4
Nitrogen-free extract	16·3	16·9	14·4	13·5	13·7	13·3	13·3	13·6	12·2	11·3	11·0	10·6
Crude fibre	5·0	5·6	1·8	1·7	5·0	4·9	2·6	2·7	0·7	0·6	3·0	3·0
Cellulose	5·8	6·4	2·3	2·2	6·4	6·3	3·2	3·5	1·0	0·9	4·1	4·2
Hexosan cellulose	4·2	4·6	1·5	1·4	4·7	4·6	2·3	2·5	0·6	0·5	3·1	3·1
Cellulosans	1·6	1·7	0·8	0·8	1·7	1·6	0·9	0·9	0·4	0·4	0·9	1·0
Pentosans-not-in-cellulose	2·5	2·6	2·2	2·0	1·9	1·8	1·7	1·7	1·4	1·3	1·3	1·1
Starch	5·1	5·1	7·4	7·4	6·2	6·2	5·1	5·1	7·4	7·4	6·2	6·2
Water drunk	126	147	94	87	92	76						

* Amounts offered; amounts of uneaten food were small and allowance has been made for them in calculating the weights of the various food constituents.

Table 4. *Digestibility of the diets given in periods I, II and III of the experiment*

	Digestibility coefficient (%)					
	Period I (initial control)		Period II (low hay)		Period III (low hay + straw pulp)	
Food constituent	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B
Dry matter	72·4	70·5	76·7	75·4	72·5	72·7
Crude protein	72·5	71·3	78·7	73·9	70·3	70·6
Ether extract	64·9	62·0	58·4	48·9	71·2	61·3
Nitrogen-free extract	81·5	80·6	84·2	83·6	80·0	79·9
Crude fibre	51·8	47·6	38·0	37·2	60·6	62·1
Cellulose	56·0	54·6	41·8	41·0	64·1	66·0
Hexosan cellulose	55·4	54·4	37·0	38·0	66·8	67·9
Cellulosans	55·2	54·8	50·9	49·2	54·4	60·9
Pentosans-not-in-cellulose	68·8	66·4	64·3	67·1	66·6	63·1
Starch	100	100	100	100	100	100
Lignin	6·8	3·7	−2·2	−2·4	−3·3	−4·2

Outstanding was the marked decrease in the digestibility of the fibrous carbohydrates with the exception of the pentosans-not-in-cellulose fraction. The fibrous carbohydrates of the low roughage-straw pulp diet (period III), again with the exception of the small pentosans-not-in-cellulose fraction, were digested to a greater extent than those of the normal hay diet (period I). There were only small between-period variations in the intake of digestible ether extract, while in periods I, II and III the mean intakes of digestible crude fibre and starch per day were 2·6, 0·6 and 3·0 lb. and 5·1, 7·4 and 6·2 lb. respectively.

Digestion in the reticulo-rumen. Results of research into the functioning of the reticulo-omasal orifice(8) suggested that digesta leaving the reticulo-rumen would not differ materially in composition from that lying adjacent to the orifice. The results of analysis of a representative sample of such digesta were used for calculating the extent of digestion in the reticulo-rumen. The tentative results obtained during the second week of each digestion trial are given in Table 5. Upwards of 70% of the total (faecal) digestion of dry matter apparently occurred in the reticulo-rumen, higher values being obtained with the low roughage and low roughage-straw pulp diets. With all three diets, over 90% of the starch digestion occurred in the reticulo-rumen. Apart from the pentosans-not-in-cellulose fraction, digestion in the reticulo-rumen accounted for 45–60% of the digestion of the fibrous carbohydrates of the normal roughage and low roughage-straw pulp diets, but small negative values (ranging from −1·9 to −13·5) were obtained for the rumen digestibility coefficients of these fractions of the low-roughage diet. When expressed as a percentage of the total digestion (Table 5) the range was −3·7 to −38·6, but the amounts concerned were very small. Considerable digestion of fibrous carbohydrates after the digesta had left the reticulo-rumen is indicated. It is noteworthy that with all the diets most of the digestion of the pentosans-not-in-cellulose fraction was accomplished in the rumen. The extent of protein breakdown in the rumen increased from mean values of 41·9% of the total digestion in the normal roughage diet to 75·4% in the diet of low roughage supplemented with straw pulp. Some of these values for digestion in the reticulo-rumen are indeed puzzling and for reasons given on p. 284 should be considered purely tentative.

Rate of passage. Table 6 shows that the rate of passage of undigested residues of hay was abnormally slow during the feeding of the diets low in hay. The initial appearance and the time required for 5% of the stained hay to be excreted are indicative of the rate of

Table 5. *Digestion in the reticulo-rumen during the second week of the collection periods (tentative values)*

	Digestion in reticulo-rumen (% of total (faecal) digestion during week 2)						Weight digested in reticulo-rumen (mean lb./day)					
	Period I		Period II		Period III		Period I		Period II		Period III	
	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B
Dry matter	78·2	62·6	79·6	74·4	79·7	82·6	16·5	13·4	13·4	12·9	13·4	14·6
Crude protein	51·6	32·2	60·4	56·0	75·0	75·9	1·9	1·2	2·0	1·8	1·6	1·8
Ether extract	75·3	53·3	71·2	72·4	62·4	72·2	0·4	0·3	0·4	0·4	0·3	0·3
Nitrogen-free extract	91·8	77·6	90·8	87·4	89·5	91·8	12·2	10·5	11·0	10·9	9·9	10·5
Crude fibre	64·3	57·0	−13·5	−19·2	53·1	59·1	1·8	1·5	−0·1	−0·1	1·5	1·8
Cellulose	59·8	46·1	−23·2	−11·6	50·0	54·7	2·0	1·5	−0·2	−0·1	1·9	2·4
Hexosan cellulose	64·9	50·4	−38·6	−12·5	55·1	56·2	1·6	1·2	−0·2	−0·1	1·6	1·8
Cellulosans	52·2	33·8	−3·7	−9·9	36·1	50·6	0·4	0·3	0·0	0·0	0·3	0·6
Pentosans-not-in-cellulose	91·1	82·2	92·9	79·7	99·5	101·6	1·6	1·4	1·2	1·2	1·2	1·2
Starch	94·3	93·0	97·5	96·8	96·6	98·5	4·6	4·6	7·3	7·4	6·1	6·2

passage of hay through the omasum, abomasum and intestines, and the time for 80% minus 5%, or the spread of the excretion curve, shows the time that residues remained in the reticulo-rumen. The diets low in hay, therefore, caused a slowing of the rate of passage in both sections of the gut. This is in agreement with our earlier experiment (4).

Table 6. *The rate of excretion of stained hay*

		Time (hr.) required for excretion of different proportions of undigested residues from stained hay					
		5% of residues*		80–5% of residues*		Initial appearance in faeces	
Period of experiment	Diet	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B
I	Initial control	22	21	60	55	16	15
II	Low hay	40	33	142	141	20	25
III	Low hay + straw pulp	37	33	115	141	22	24

* For explanation of these indices of the rate of excretion see p. 276.

Table 7. *Mean time spent by cows in different daily activities*

	Period I (initial control)		Period II (low hay)		Period III (low hay + straw pulp)	
Activity	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B
			(min.)			
Eating	240	252	209	204	141	199
Standing:						
Ruminating	193	357	119	18	72	20
Resting	308	413	543	582	492	492
Total standing	741	1022	871	804	705	711
Lying:						
Ruminating	401	165	124	99	206	134
Resting	298	253	445	537	529	595
Total lying	699	418	569	636	735	729
Total ruminating	594	522	243	117	278	154
Proportion of day (%)						
Standing	51·5	71·0	60·5	55·8	49·0	49·4
Lying	48·5	29·0	39·5	44·2	51·0	50·6
Ruminating	41·2	36·2	16·9	8·1	19·3	10·7
Days recorded	7	7	8	8	7	8

Behaviour of the cows. From records of reticular movements it is possible to determine the time spent by the cows in each of the major types of activity (9). The main changes observed (Table 7) were a marked reduction in the time spent ruminating and a smaller reduction in the time spent eating, with consequent increases in the time spent resting. The time spent ruminating was reduced in cow A from 41·2% of the day in the control period to 16·9 and 19·3%, and in cow B from 36·2% in the control period to 8·1 and 10·7% in periods II and III respectively. The large proportion of the time spent standing by cow B in the first period was due to rubbing by the harness.

Rate of reticulo-ruminal movements. Using the rate of contraction of the reticulum as an index of reticulo-ruminal motility it was found (Table 8) that the usual general trends in rates of motility (9) existed with both the normal diet and those low in hay. Thus the most rapid rates of contraction were found during eating, and rates were slower during lying than standing, except in cow B, during the first period. The rate of contraction was more

rapid during rumination than during resting, an effect opposite to that recorded in our earlier report (9) and possibly due to the much smaller proportions of hay given in the present experiments. Cow B (Halora) was used also in the earlier experiments where she was cow Y.

Reduction in the roughage content of the diet had little effect on the rate of reticular contraction during eating, but during other types of activity the rate of contraction tended to be less rapid; this was very marked when the cows were lying resting or, except for cow A during period III, ruminating. During periods of lying and resting the reticulum of cow B sometimes contracted at the exceptionally slow rate of only one or two contractions in 20 min.

Table 8. *Mean rate of reticular movements* (*double contractions/min.*)

	Period I (initial control)		Period II (low hay)		Period III (low hay + straw pulp)	
Activity	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B
Eating	1·47	1·46	1·43	1·26	1·45	1·32
Standing:						
Ruminating	1·22	0·92	1·13	0·97	1·17	0·95
Resting	1·20	0·86	0·94	0·84	0·97	0·93
Lying:						
Ruminating	1·10	1·11	0·98	0·96	1·13	0·95
Resting	1·02	1·08	0·70	0·50	0·80	0·74

The amount of digesta in the reticulo-rumen. Reduction in the proportion of hay in the diet caused marked falls in the total weight of digesta and in the weight of dry matter in the reticulo-rumen (Table 9). The total weight and weight of dry matter present in periods II and III were approximately 70% of the amount present in the initial control period. By comparison, the dry-matter intake fell to 73 and 80% in periods II and III respectively. In previous experiments the animals lost weight when given diets low in hay, and this experiment shows that losses in body weight of up to 55 lb. may be due merely to changes in the amount of digesta in the reticulo-rumen. In the present experiment the animals lost approximately 60–80 lb. during the period of low hay feeding, but this loss was regained in the next period, and the weights of the cows in the final control period suggest that there was no real overall loss in body weight.

Although the single complete emptyings showed that there was little change in the dry-matter percentage of the total digesta, mean values for the several samplings of digesta in the ventral sac and in the reticulum showed a raised dry-matter content with the diets low in hay. This effect is similar to that produced by finely grinding all the hay in the diet.

The pH *and concentration of organic acids in the reticulo-rumen.* The hourly variations of pH and the concentration of total volatile acid in the reticulo-rumen of cow A throughout a 24 hr. period during the feeding of each diet are shown in Fig. 2. Similar fluctuations were observed with cow B, and the values for both cows are summarized in Table 10. In each period the pH and volatile acid concentration fluctuated inversely. The increase in concentration of total volatile acid after feeding, and the subsequent decrease as fermentation declined, were greater for the low hay and low hay + straw pulp diets than for those containing normal amounts of hay. Peak values were higher and minimum values lower, and the buffering capacity of the rumen was also reduced.

Table 9. *Amount and dry-matter content of reticulo-ruminal digesta at* 12 *noon and weight of cows*

Period of experiment	Diet	Weight (lb.)				Dry matter (%)								Weight of cow (lb.)	
		Total contents		Dry matter		Total contents		Dorsal sac*		Ventral sac*		Reticulum*			
		Cow A	Cow B	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B
I	Initial control	168·7	164·4	22·4	22·4	13·3	13·6	14·3	13·9	10·5	10·5	6·5	7·1	1217	1120
II	Low hay	117·5	109·0	16·1	14·8	13·8	13·6	15·2	14·9	13·2	12·9	10·6	9·8	1156	1043
III	Low hay + straw pulp	124·2	123·9	16·3	16·6	13·1	13·4	14·0	14·8	13·2	13·4	11·9	12·1	1214	1131
V	Final control	186·1 (166·1)†	159·1	21·7	20·7	11·7 (13·1)†	13·0	—	—	—	—	—	—	1290	1153

* Based on daily samples for 7 days; other values based on single emptyings of complete organ.
† Immediately before this emptying in the final control period cow A drank 20 lb. water. Values in parenthesis show the effect of discounting this intake.

Table 10. *The* pH *and volatile fatty acid content of digesta in the reticulo-rumen*

Period of experiment	Diet	Range of pH over 24 hr.		Range of total volatile acid concentration over 24 hr. (m.equiv./100 ml. rumen liquor)		Individual volatile fatty acids (% by weight of total at 12 noon)							
						Acetic		Propionic		Butyric		Higher acids*	
		Cow A	Cow B	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B
I	Initial control	5·4–6·9	5·5–7·0	10·6–18·3	10·6–15·9	49·2	50·8	25·1	25·7	15·2	17·2	10·5	6·3
II	Low hay	4·3–7·0	4·9–6·8	5·6–20·0	7·8–16·6	33·5	33·3	39·7	36·8	9·1	12·8	17·7	17·1
III	Low hay + straw pulp	5·0–6·8	4·9–6·3	7·8–20·0	11·4–18·8	38·1	40·6	31·8	29·6	16·8	16·3	13·3	13·5
V	Final control	5·9–6·9	5·9–7·2	9·4–16·1	7·7–14·6	59·9	59·6	20·7	21·6	13·7	12·8	5·7	6·0

* Expressed as valeric acid.

Appreciable concentrations of lactic acid were found in the reticulo-rumen shortly after feeding, except with the normal hay diet in period V. The lactic acid rapidly disappeared, only trace values being present about 4 hr. after feeding. With cow A peak values of 165, 220 and 120 mg./100 ml. rumen liquor were found in periods I, II and III respectively, the values for cow B being rather lower.

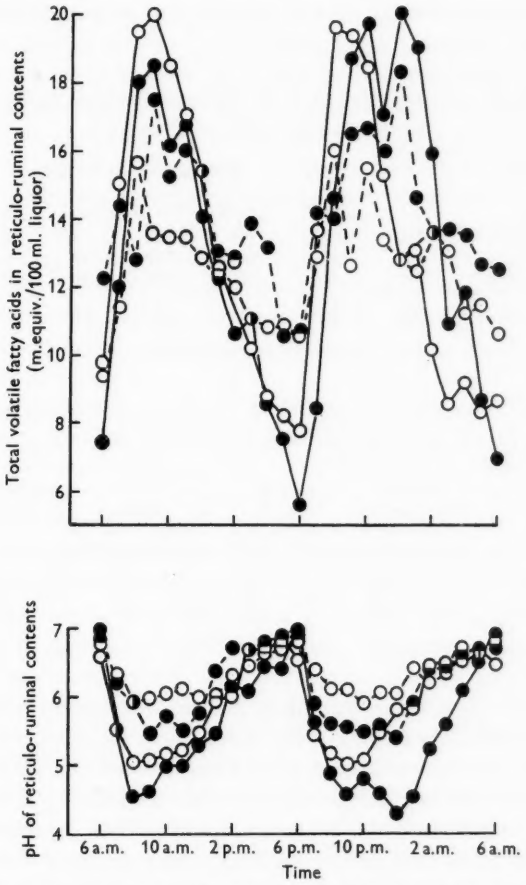

Fig. 2. The total volatile fatty acids and the pH of the reticulo-ruminal contents of cow A throughout a 24 hr. period. Values are shown for the control diets, periods I, ● - - - ●, and V, ○ - - - ○, and for the two diets low in hay, periods II, ●——●, and III (supplemented with straw pulp), ○——○. The cow was fed at 6 a.m. and 6 p.m.

The relative proportions of the volatile fatty acids. Mean values for cows A and B for acetic acid (Table 10) fell from 50·0 and 59·8% of the total volatile fatty acids in periods I and V to 33·4 and 39·4 in periods II and III respectively, while those for propionic acid rose from 25·4 and 21·2 to 38·2 and 30·7. The proportion of acids higher than butyric, calculated as valeric acid, was also higher in periods II and III than in periods I and V. In contrast the values for butyric were variable, being lower in period II than in periods

I and V, but higher in period III. The agreement between the values for the two cows was good.

The weights of individual fatty acids in the reticulo-rumen. The weights of the different acids present in the reticulo-rumen at 12 noon are given in Table 11. Mean values for acetic acid during the control periods (I and V) were 314 and 328 g., while in periods II and III the values were much lower, 146 and 221 g. respectively. It is interesting that until period V there was little variation in the weight of propionic acid present with the different diets.

Table 11. *The weights (g.) of the individual volatile fatty acids in the reticulo-rumen at 12 noon*

Period of experiment	Diet	Acetic		Propionic		Butyric		Higher*	
		Cow A	Cow B	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B
I	Initial control	350	279	179	141	108	95	75	34
II	Low hay	157	136	186	151	42	53	83	70
III	Low hay + straw pulp	208	233	173	170	92	94	72	78
V	Final control	342	313	118	113	80	67	32	31

* Expressed as valeric acid.

Table 12. *The concentration (mg./100 ml.) of glucose and serum lipids in blood from the jugular vein (each value is the mean for two or four samples)*

Period	Diet	Total cholesterol (*a*)		Phospholipids (*b*)		Total fatty acids (*c*) (free and esterified)		Total lipids (*a* + *b* + *c*)		Glucose	
		Cow A	Cow B	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B	Cow A	Cow B
I	Initial control	170	201	161	189	103	159	434	549	51·9	50·6
II	Low hay	84	104	61	56	68	72	213	232	55·0	54·3
III	Low hay + straw pulp	167	202	72	178	111	122	350	502	53·5	55·2
V	Final control	155	160	97	107	98	108	350	375	—	—

Blood constituents. Mean values for the concentration of glucose and serum lipids in jugular vein blood during the feeding of the various diets are given in Table 12. The concentration of blood glucose was normal, there being no variation with diet. The change from the normal to the low hay diet (period II) was accompanied by a decrease of approximately 50% in the concentration of all serum lipid fractions. Apart from the phospholipid content of samples from Dorrit 15, there was a recovery in all values on the addition of straw pulp to the diet, there being no further increase when the normal hay diet was restored in period V.

DISCUSSION

In the two fistulated cows the falls in milk-fat percentage were larger than those in our earlier experiments and the changes in the intake of digestible nutrients and in the digestibility coefficients followed the trends observed previously. In addition, it was found that the milk-fat content remained low in spite of the addition to the diet of sufficient macerated straw pulp to raise the daily intake of digestible crude fibre above the intake in the control periods. This confirms the suggestion (20) that a high intake of crude fibre, in a finely divided form lacking the physical property of fibrousness, will not prevent

falls in milk-fat content. The critical features of the diets with which the falls in milk-fat content occurred were again a high content of digestible starch—the mean daily intake by the cows was 7·4 and 6·2 lb. in periods II and III respectively—when the diet had little of the physical property of fibrousness. The initial control period—when the mean intake of digestible starch was 5·1 lb. daily and the milk-fat percentage normal—provides good proof that with ample fibrousness high intakes of starch do not cause falls in milk-fat content.

Further confirmation that the effects of the dietary changes were greater in this than in our earlier experiments is provided by the extremely slow rate of passage of hay and the changes in the yield and composition of the milk fat. It is estimated that the cows produced only 38–49% of the fat they would have produced if the control diet had been given continuously. Decreases in the Reichert value of the butterfat and increases in the iodine value were more pronounced than previously (1). In agreement with one of our earlier experiments (4) there was a marked increase in the solids-not-fat content of the milk at the time of the low milk-fat content.

Many observations confirmed, therefore, that these cows were suitable subjects for a more detailed study of the conditions associated with the falls in milk-fat content. Accordingly, especial attention was given to the physical and biochemical conditions found in the reticulo-rumen.

Reticulo-ruminal movements

The main change in behaviour when the cows received the diets low in hay was a marked reduction in the time spent ruminating. As with ground hay (8), the straw pulp lacked the physical properties necessary to initiate the regurgitation reflex. Except during eating, the rate of reticular contraction tended to be slower than normal with the diets low in hay; this was especially marked and at times the rate was quite abnormally slow when the animals were lying down. It suggests that reticulo-ruminal movements in general were more sluggish and that in consequence the amount of mixing of digesta may have been reduced. During the two periods of diets low in hay the amounts of digesta present in the reticulo-rumen were approximately 70% of those found in the initial control period. Because of the reduced volume and the lack of hay the digesta from diets low in hay were very easily stirred, and the fewer contractions may have been adequate to mix the digesta. Support for this suggestion was provided by the slow excretion of stained particles. The prolonged excretion curves could have been produced only if newly ingested food were thoroughly mixed with the digesta already present. There can have been little tendency for newly ingested food to push out the residues of previous meals before mixing took place, because this would have reduced the spread of the excretion curves; this easy accommodation of newly ingested food was clearly made possible by the small amount of digesta present. The reduced amount of rumination may also have been partly responsible for the slow excretion. The amount of mixing taking place in short periods of time, rather than in the longer periods reflected in measurements of the rate of passage, may have been of considerable importance in determining how rapidly the products of digestion were absorbed from the reticulo-rumen. It should therefore be emphasized that although it is not clear to what extent the efficiency of mixing changed when the proportion of hay in the diet was reduced, it is probable that the circulation of digesta within the reticulo-rumen was slower than with normal diets.

Digestion in the reticulo-rumen

Recent experiments suggest that the passage of digesta from the reticulo-rumen is accelerated during eating (Balch, unpublished). As the method of sampling used in the present experiment did not take account of this acceleration high values may have been recorded for the digestion in the reticulo-rumen of the constituents of concentrates and low values for those of hay. In both cows small negative values were recorded for the digestibility, in the reticulo-rumen, of the fibrous carbohydrates of the diet low in hay (period II). With the increased intake of starch, which is known to depress the digestion of fibrous carbohydrates (see review by Head (21)), a low digestibility coefficient was to be expected: that the values proved to be negative is attributed to the method of sampling and to the very small amount of these constituents involved (see Table 5). The technique of estimating digestion in the reticulo-rumen and the interpretation of the results are to be considered in a further publication. It is, however, likely that the observed depression in the total (faecal) digestion of fibrous carbohydrates in period II was due to changes taking place in the reticulo-rumen.

It should be noted that the total weight of carbohydrate apparently digested in the reticulo-rumen with the two diets low in hay was greater than with the control diet. The weight of starch in the diets given during periods I, II and III, upwards of 90 % of which was recorded as digested in the reticulo-rumen, was sufficient to rank these as diets high in starch. It is, therefore, not surprising that the proportion of fibre digested in the remainder of the gut was always substantial although the amounts concerned were again small.

Since very little starch appeared to pass undigested from the rumen, there was no appreciable difference between diets in the proportion of carbohydrates digested by microbial action. It is known that the types and numbers of micro-organisms which become established are influenced by the chemical composition of the diet and especially by the presence in the diet of maize (22) or flaked maize (23), and it seems probable that the flora may also be modified by changes in the physical conditions and especially in the pH in the rumen. Diets from which milk of low fat content was produced caused marked physical changes in the reticulo-rumen.

Production of volatile fatty acids

Accompanying these physical changes, chemical changes in the course of fermentation were observed, the salient features being the more rapid increase and subsequent decrease of total volatile fatty acid concentration after feeding, the maintenance of low pH values for long periods of time and the changes in the relative proportions of the volatile fatty acids. The only consistent changes in the end-products of carbohydrate digestion in the rumen at the time of the secretion of milk of low fat content were the decrease in the proportion of acetic acid and the increase in that of propionic acid. Stoddard, Allen & Peterson (24) and Tyznick & Allen (25) also reported a lowering of the proportion of acetic acid in the rumen with low roughage diets, but no marked change was found by McClymont (26).

The proportion of acetic acid fell from about 50 % of the total volatile acids present in the reticulo-rumen during the control period to about 33 % in period II, the proportion

of propionic acid rising from about 25 to 38%. This finding, when considered in conjunction with the decrease which occurred at the same time in the volume of reticulo-ruminal contents, indicates a considerable decrease in the weight of acetic acid present, whereas the weight of propionic acid remained at about the same level in each period of the experiment. It is interesting to note that in period III, in which the cows received straw pulp, values for acetic acid were intermediate between those of period II and the control period, although the milk-fat percentage did not rise.

While it is not entirely safe to assume that the observation of a decreased residual weight and concentration of a substance in the reticulo-rumen necessarily implies that the amounts actually absorbed through the wall of the reticulo-rumen have decreased, it does appear probable that with the diets low in roughage less acetic acid was absorbed than with the control diets. Because acetic acid, but not propionic acid, is known to be a precursor of the saturated fatty acid fraction of the milk fat of ruminants (27), it is not unreasonable to suggest that the cause of the low milk-fat percentage was a deficiency of acetic acid. The claims of Allen and colleagues (24,25) to have raised the fat percentage of milk from cows receiving diets low in roughage by the administration of acetate supports this hypothesis, and similar preliminary observations recently made at this Institute are in agreement (Balch & Balch, unpublished data).

McClymont (28) observed a high positive correlation between concentrations of volatile fatty acids in the rumen and arterial levels of acetic acid. Because of the greater range of concentration of volatile fatty acids in the rumen owing to the rapid fermentation of carbohydrates, it is probable that with the low-roughage diets there was initially a more rapid, and subsequently a slower, absorption of acetic acid than with the normal diets. Acetate injected into a goat was rapidly oxidized, 80% being converted to carbon dioxide in 6 hr. (29). If a similar rapid catabolism occurs with cows (indicating the lack of an efficient storage of acetate), the immediate capacity of the anabolic reactions involving acetate might be exceeded at peak absorption, the excess being wastefully oxidized. This would aggravate the deficiency in the production of acetic acid. With diets which depressed milk-fat percentages and Reichert values to about the extent observed in cows A and B, McClymont (28), while concluding that acetic acid is a normal and major metabolite of the mammary gland and a precursor of the short-chain acids, could demonstrate no association between acetic acid uptake and the proportion of lower fatty acids in the milk fat. In his experiments arterial levels of acetic acid and mammary arterio-venous differences in acetic acid were of the same order for both low roughage-high concentrate and normal diets. In this study McClymont (28) gave no comparison of the proportions of the various volatile fatty acids in the rumen with normal and with low-roughage diets, but in earlier work he (26) did not observe the increase in the proportion of propionic acid and decrease in the proportion of acetic acid observed in the present experiment. There is, therefore, an unexplained difference between the results here reported and those given by McClymont. The main difference in diets used appears to be that in all the present series of experiments flaked maize formed a large proportion of the concentrates, whereas the concentrates used by McClymont contained no maize. With lambs, Phillipson (30) also found that diets high in flaked maize produced an unusually low ratio of acetic to propionic acids in the rumen.

Composition of milk fat

The mechanism of milk fatty acid synthesis in the cow is probably similar to that worked out for the goat by Popják, Folley, French and their associates (29) where acetate contributes mainly to the synthesis of saturated acids containing up to 16 carbon atoms and especially of the volatile acids. During the secretion of milk of low fat content, the fat contained a lowered proportion of such short-chain saturated acids. This has also been found by other workers (24,25,31). A similar decrease was found by Smith & Dastur (32) on fasting cows when there would be a marked decrease in absorption of acetate from the reticulo-rumen. Folley & Malpress (33), Mann & Shaw (34) and McClymont (28), however, failed to increase such lowered Reichert values by the intravenous or intraruminal infusion of acetate, although in McClymont's experiments on fasting, normal acetate levels were maintained in arterial blood and considerable uptake of acetate by the mammary gland was demonstrated. McClymont suggested that the content of short-chain acids in ruminant milk fat is determined by the rate of secretion of the fat. He postulated that unless the short-chain acids are esterified and secreted into the milk they will be rapidly converted to the next higher acid. The results of Popják, French, Hunter & Martin (35), indicating that the 'pools' of short-chain acids in the mammary gland are small, support this view. Although the lowered Reichert values cannot be taken as unequivocal evidence of lowered synthesis, the concurrent changes in iodine value were consistent with the theory that the decrease in milk-fat content was mainly due to a decrease in the secretion of glycerides containing saturated fatty acids, the majority of which could arise by synthesis from acetate.

Saturated acids containing 14 or fewer carbon atoms normally account for only some 29%, on a molar basis, of the total fatty acids (36), yet the diets low in hay caused a reduction of over 50% in the amount of fat produced. This reduction clearly cannot have been due entirely to a decrease in the secretion of short-chain acids which can be synthesized from acetate, and calculations made from the iodine values also suggest that there was a decrease in the secretion of unsaturated acids which probably arise from blood fat rather than by synthesis from acetate. Although a marked decrease in the concentration of blood lipids was observed with the diet low in hay, the concentration returned to normal, without a recovery in milk-fat percentage, with the diet which was low in hay but contained straw pulp. This suggests that a shortage of unsaturated acids was not the critical factor in the occurrence of the low milk-fat percentages. In view of the observation that the amount of ether extract digested daily remained at 0·4–0·5 lb. in the initial control period and with both of these diets low in hay, no shortage of unsaturated acids was to be expected.

Considerable energy is required by the mammary gland for the formation and secretion of milk. In the ruminant such energy can be obtained by the oxidation of acetate. A decreased supply of acetate would not only depress the synthesis of milk fatty acids but would also reduce the amount available for oxidation. Such a decrease in available energy could lead to a lowered secretion of the components of milk fat which are not formed by synthesis from acetate. At the time of the secretion of milk of low fat content there is, admittedly, no marked decrease in the volume of milk secreted and occasionally there has been an increase in its content of non-fatty solids. It is, however, by no means certain that a decreased supply of acetate would be distributed evenly among the diverse

metabolic systems of the mammary gland. Owing to the utilization of acetate by the mammary gland either for energy or for synthesis, the depression in milk-fat content may well be caused by a decrease in the supply of acetate to the mammary gland. The effect of supplementing diets low in hay and high in concentrates with sodium acetate and sodium propionate will be dealt with in a later paper.

SUMMARY

1. Two Shorthorn cows with rumen fistulas were used to investigate the effect of diets high in concentrates and low in hay on the physical and biochemical processes of the reticulo-rumen.

2. During the initial control period of the investigation the cows received daily 16 lb. hay and 20 lb. concentrates (flaked maize 50%, weatings 35%, decorticated ground-nut cake 15%) and during the final control period 18 lb. hay and 10 lb. concentrates. There were three intervening experimental periods, in each of which the cows received 2 lb. hay daily. In the first experimental period they received, in addition to the hay, 24 lb. concentrates; in the second, 20 lb. concentrates and 5 lb. of dried delignified straw pulp in a finely macerated form; and in the third, 20 lb. concentrates. The experiment lasted 27 weeks.

3. In the experimental periods the mean milk-fat percentage remained below 2·0 for 10 weeks, whereas the mean value for the control periods was about 3·5. Addition of the straw pulp to the diet low in hay brought about no recovery in milk-fat percentage. It is calculated that in the two cows the losses in the yield of fat were 62·2 and 51·2% in the first experimental period and 62·5 and 58·7% in the second. In the experimental periods there were increases in the milk solids-not-fat percentage amounting in the two cows to upwards of 0·75 and 0·40 respectively. The composition of the butterfat was influenced by the diets low in hay, the main changes being a marked fall in the Reichert value, and a rise in the iodine value.

4. Digestibility trials showed that in the initial control period and the first and second experimental periods the mean daily intake of digestible crude fibre was 2·6, 0·6 and 3·0 lb. respectively. A tentative estimate of the extent of digestion in the reticulo-rumen, based on the lignin-ratio method, showed that a marked depression in values for the digestibility of the cellulosic constituents of the diet given during the first experimental period took place in the reticulo-rumen. In all experimental periods, even the first, the digestion of starch in the reticulo-rumen was virtually complete, only traces passing undigested to the remainder of the gut. In these three periods the mean daily intake of ether extract remained at 0·4–0·5 lb.

5. The falls in milk-fat content were found only with diets which contained large amounts of starch (6·2 or 7·4 lb./day) and had little of the physical property of fibrousness. A moderately high starch intake (5·1 lb./day) in the presence of 16 lb. hay caused no fall, neither did the macerated straw pulp bring about a recovery during the second experimental period.

6. The changes in diet caused no change in blood glucose concentration, but in the first experimental period there was a decrease of approximately 50% in the concentration of all serum lipid fractions. The recovery of these values in the second experimental period suggests that the fall in milk-fat content was not primarily due to a decrease in serum lipid concentration.

7. The main change in the behaviour of the cows in the experimental periods was a marked reduction in the time spent ruminating. The diets low in hay also caused a considerable slowing of the rate of contraction of the reticulum (except when the animals were eating) and especially when they were lying down, suggesting that at certain times of day the motility of the reticulo-rumen was sluggish. The reticulo-rumen contained less digesta than with the control diets, and particles of hay tended to remain there for an abnormally long time.

8. With the diets low in hay the peak concentration of the total volatile fatty acids in the reticulo-rumen was higher, and the pH lower, than in the control periods, but the weights of acetate present in the reticulo-rumen were much reduced whereas the weights of propionate remained at about the same level until the final control period. It therefore seems probable that the amount of acetic acid absorbed from the reticulo-rumen is depressed by diets low in hay and high in concentrates and that, as acetate is utilized as a source of energy and of milk fat by the mammary gland, the resulting fall in milk-fat percentage may be due to a reduction in the amount of acetate available to the gland.

We wish to acknowledge the advice of the late Dr T. H. French in the planning of this experiment and our thanks are due to Miss H. R. Chapman and the staff of the Experimental Dairy for preparing the butter samples. We also wish to thank Messrs D. G. Kemp and F. Leaver for their care of the cows and Miss M. H. Booty, Mr D. Millard, Mrs R. Winder and Mr A. W. Wagstaff who carried out much of the analysis.

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593. INTRAMAMMARY PRESSURE CHANGES IN THE LACTATING COW

I. CHANGES DURING THE MILKING PROCESS

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(With 4 Figures)

Current views on the nature of the pressure changes which occur within the udder of the cow at milking time have been greatly influenced by the pressure graph published by Tgetgel (1) showing the increase in intramammary pressure between milkings followed by the rapid changes during the act of milking. This graph has been confused with the changes thought to occur after milk ejection in an unmilked udder, and there is still considerable uncertainty as to the nature of the changes following milk ejection both when the cow is not milked and when milk is withdrawn. Miller & Peterson (2) state that 'the ejection pressure subsides after a time and the milk is not all forced down when milking is actually done'. Experimental evidence of such pressure changes is not given. Peeters, Coussens & Oyaert (3) show the effects of injecting pitocin into the perfused udder. Their pressure responses show a very rapid rise followed by a slow fall which would appear to approach a limiting pressure considerably above the level before injection.

Earlier experiments at this Station (4), in which the lactating sow was used, have shown that in this case oxytocin produces a rapid rise in pressure followed immediately by a fall, which within a short time returns to the initial level. This is quite different from the pressure curve found by Peeters *et al.* for the cow. The present study shows the nature of the pressure changes which occurred following 'let-down' in Jersey cows.

There are many difficulties involved in udder-pressure measurement in the cow. Perhaps the greatest difficulty arises from the fact that small movements of the hind legs may cause large changes in intramammary pressure. It was necessary, therefore, to select cows which would stand without moving for 10–15 min. periods. The cow used most frequently was so well conditioned that milk ejection occurred when the udder was being washed prior to inserting the cannula.

THE APPARATUS

All measurements were made using the mercury manometer described earlier (4). Two manometers were used, and as the laboratory possesses only one recorder, the pressure heads were switched in alternately. The recorder used was an Elliotronic strip-chart recorder, and the d.c. current through the manometer spiral was obtained from two 1·5 V. telephone dry cells with series resistors to limit the voltage drop to a maximum of 10 mV. with the mercury at zero pressure.

METHODS

Two fore-quarters were used, one being cannulated with an ordinary teat siphon connected directly to a manometer, the other with a teat siphon fitted with a by-pass tap which enabled the operator to draw milk from the quarter concerned. This siphon was connected to the second manometer. As soon as the siphons were fixed in place the recorder was switched from one manometer to the other every 15 sec. (except when the udder was being stimulated when longer intervals were used). One quarter was drained via the two-way siphon during the 15 sec. period when the undrained quarter was being recorded. In this way on one strip chart it was possible to record alternately the pressure within a milked and an unmilked quarter. Movements affecting the pressure within the udder may be seen as steps in the pressure graphs. Owing to the relatively constant nature of the pressure within the unmilked quarter, this may be used as a 'control' in determining the changes occurring during the milking-out process. The draining cannula removed milk at a rate similar to normal milking, and because it was operating only one-half of the time, resulted in a total 'milking' time of double the normal time. When milk flow ceased the udder was stimulated by rubbing and the stripping of the hind teats. This resulted in a 'second let-down'.

The manometers were placed at such a position that they read zero when the cannulae were held at the level of the cow's teats, thus giving the true intra-teat pressure. Connexion between teat and manometer was effected with rubber tubes containing sterile saline, a small amount of which was allowed to run into the udder to ensure the patency of the cannulae.

Note on Figures 3 *and* 4

In order to show detail of the pressure changes at certain parts of the graph, an enlarged time scale and continuous recording was used. Fig. 3 shows two enlarged portions, the duration of which is noted on the time scale. The first shows variations in pressure as the udder is massaged, the second the nature of the pressure rise during a 'second let-down'. In Fig. 4 a similar second pressure rise is shown in detail.

RESULTS

In all cases the cow had let her milk down before the cannulae were inserted so that the pressure change due to milk ejection could not be recorded. Figs. 1–4 show typical results. All records made are of this type, so that the reproduction of more would add nothing to this report.

Fig. 1 shows a small fall in pressure within the undrained quarter. Two steps occur which were associated with the movement of the cow. The 'second let-down' was associated with a small rise and fall in pressure. The total yield of milk from the drained quarter (3·1 lb.) was normal.

Fig. 2 is similar to Fig. 1. The fall in pressure in the unmilked quarter is small, while the 'second let-down' gives a characteristic small rise and fall. This second pressure wave in the milked quarter is not associated with any obvious change in the unmilked gland.

In the case of Fig. 3 an attempt was made to plot the course of the rise in pressure due to the 'second let-down' in more detail. There is a fairly rapid rise in pressure followed by a characteristic fall as the quarter is drained. In both Figs. 2 and 3 the milk ceased to

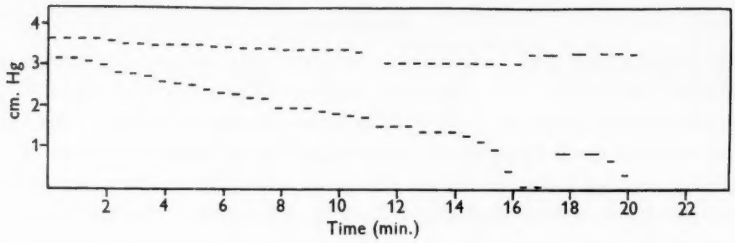

Fig. 1

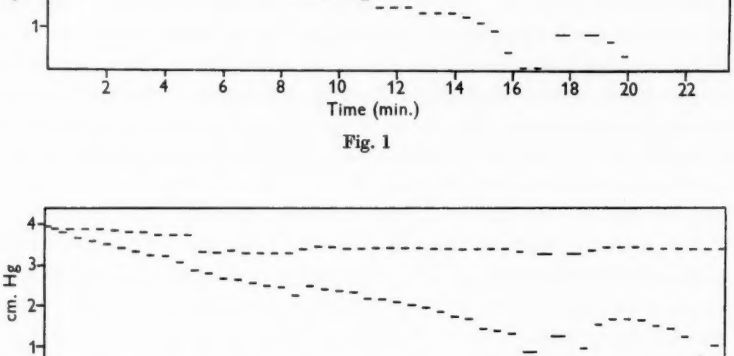

Fig. 2

Fig. 3

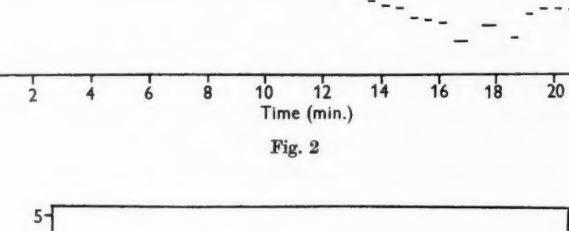

Fig. 4

drain before the pressure reached zero. This is probably due to the collapsing tissue within the teat closing around the cannula.

The graph shown in Fig. 4 is the product of an attempt to stimulate the cow to give a 'second let-down' after 4½ min. No change occurred. When the quarter ceased to drain the cow was again stimulated, producing the pressure rise shown near the end of the graph.

In all experiments so far carried out the pressure rise due to a 'second let-down' in the milked-out quarter caused no change in the corresponding unmilked gland. Further, the total yield of milk from the drained quarter was normal in all cases, showing that the long-drawn-out 'milking' involving a second release of milk is just as efficient as a faster milking involving a single 'let-down'. This corresponds with earlier results (5) obtained by partial inhibition of milk ejection followed by a double milking procedure.

DISCUSSION

The characteristics of the pressure graphs obtained from cow and sow are quite different. The latter corresponds to the view that the posterior lobe hormones cause a sudden contraction of the myoepithelium (6,7) followed by a slower relaxation. This is associated with a rapid transfer of milk from the alveoli into the ducts and back again when the gland is not milked. In the case of the cow the pressure rise due to milk ejection is followed by a very slow fall. If a cow is stimulated, allowed to stand for a short time (10 min.), and finally milked without additional stimulation, only a fraction of the normal yield is obtained, showing that while the pressure may not fall due to the milk ejection mechanism going out of action, something happens which prevents the draining of all but the larger ducts. Either one or both of two mechanisms may operate. Peeters (3) suggests the existence of 'valves which form the transition from the great to the small milk ducts'. Another possibility (8) is that during the period of action of the posterior lobe hormone the contraction of myoepithelium along the small ducts results in the latter opening against the pressure of milk within the alveoli. This 'duct opening' action would result in the pressure within the large ducts coming to equilibrium with that within the alveoli, while the myoepithelium remained contracted, but would close off the ducts from the alveoli when contraction ceased. The final explanation will depend on histological evidence for valves within the duct system and/or a demonstration that there is a difference between the cow and the sow in the way in which the contracting myoepithelial fibres may affect the ductules.

The pressure changes within the milked-out quarters show the sort of trend to be expected during the draining of a partly elastic bag. The pressure rise and fall following a second stimulation is convincing additional evidence for the view (5) that the normal dairy cow may respond to stimulation within a short time after the commencement of milking with a second release of milk-ejection hormone. What is quite clear is that whatever the difference in anatomy between the cow and the sow, the ways in which intramammary pressures change in response to the action of the milk-ejection hormone are quite different.

SUMMARY

Using an electrical pressure-recording system simultaneous pressure records have been made in both the milked and unmilked quarters of a dairy cow. The unmilked quarters showed a very slow fall in pressure, while, as would be expected, the milked quarter showed

a steady pressure fall. Re-stimulation some time after the beginning of the experiment resulted in a rise and fall in pressure within the milked quarters, but no change when the quarter was not milked.

The writer is indebted to Mr R. D. J. Parkinson for technical assistance during the experiments described.

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594. THE EFFECT OF HEAT TREATMENT ON THE LIPOLYTIC FLORA OF CREAM

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In the dairy industry pasteurization of cream is a normal practice prior to buttermaking, in order to improve the keeping quality of the finished product. As there are no regulations governing the heat treatment of cream, the time/temperature combinations used vary widely in practice, but the two commonest processes are those based on the pasteurization of milk—namely, the 'holder' process of approximately 63° C. for 30 min., which is most useful for small amounts of cream, and the 'flash' or H.T.S.T. process, in which a temperature of 73° C. held for periods of 15 sec. allows the process to be made continuous with a saving of time, space and labour.

Recently legislation has been altered in this country to allow the retail sale of various kinds of cream, and in most cases some form of heat treatment is applied to these products to make them safe and extend their keeping time. In a fat-rich product like cream one of the most obvious forms of deterioration will be acid rancidity. Souring can also occur, but, since most of the lactose-fermenting bacteria are killed by pasteurization and many thermoduric bacteria have lipolytic powers, acid rancidity is perhaps the most important fault in heat-treated cream. Information concerning the survival of lipolytic organisms in heated cream will thus be important as regards the keeping quality of cream and of its product, butter.

Whilst there is much published information on the organisms in milk which survive pasteurization, very little of this deals with their lipolytic nature. The thermoduric flora of cream has also received scant attention. Hammer (1) in 1914 studied the effect of 'flash' and 'hold' pasteurization of cream prior to buttermaking. He made no specific count of lipolytic organisms but concluded that the keeping quality of the butter was prolonged by cream pasteurization, although as great differences were found between different methods of heat treatment as between raw and heated cream.

The standard method in Britain for the examination of butter for lipolytic flora (British Standards Institution (3)) uses tributyrin agar as the differential medium. This medium had disadvantages (Jones & Richards (4)), and other media of greater discrimination have been described. Richards & Sadek (5) showed that of the fatty acids recovered from rancid butter about 75% were oleic-like unsaturated acids. On this basis, and in view of the advantage of a chemically defined substrate, triolein was tried and found to give excellent results (Jones & Richards (4); Sadek (6)).

In the present work we have made counts of the tributyrinolytic organisms of raw and pasteurized cream according to the standard method. A representative number of these tributyrin-splitting organisms has been isolated for characterization and tested for ability to hydrolyse triolein as a measure of true lipolytic character.

EXPERIMENTAL METHOD

Preparation of cream samples. All samples were adjusted to constant fat content by receiving from the separator both cream and separated milk from the same milk sample and adding separated milk to the cream as required to bring it to 30% fat content (by the method of B.S.S. 696 Part 2 (2)). The standardized cream was pipetted aseptically in 5 ml. amounts in $6 \times \frac{5}{8}$ in. test-tubes, taking great care not to contaminate the upper walls of the tube with cream. Where slight contamination occurred the patch of cream was charred by heating the tube in the flame.

Heat treatment. In the low-temperature process the tubes were placed in a water-bath at 65° C. and held there for 35 min. It had been previously observed that 5 ml. of cream in a test-tube reached 65° C. in about 5 min. when immersed in the water-bath. For the high-temperature process a water-bath at 71° C. was used. In this case preliminary experiment showed that 5 ml. of cream in a test-tube took 270 sec. to reach 71° C. Pasteurization was therefore carried out with an immersion time of 284 sec. As it took 1 sec. to transfer the sample from the bath to chilling water, the holding period was thus 15 sec. It is recognized that this method appears somewhat crude and that its effect is not necessarily the same as that obtained in commercial H.T.S.T. practice in a continuous flow device; this point will be commented on in the discussion. Laboratory high-temperature short-time treatments of batch samples are notoriously difficult to standardize, but it is likely that the treatments from batch to batch did not vary greatly, and since the results of laboratory H.T.S.T. processes on milk give much the same microbiological picture as in commercially treated samples, it is possible that this also largely applies to cream. After heat treatment samples were cooled by immediate immersion in running cold water at about 12° C. for 10 min.

Colony counts. All cream samples were serially diluted in quarter-strength Ringer solution, plated with tributyrin agar (B.S.S. 895 1940 (3)) and incubated for 4 days at 30° C. Tributyrinolytic colonies were counted, using a hand lens, a positive standard being a clear zone greater than 0·5 mm. around the colony. All the tributyrin-splitting colonies from the highest dilution plate, and, if not numerous enough, colonies from the preceding plate in the series, were picked into yeast extract peptone broth and thence characterized as far as possible by morphology, Gram reaction, and reactions in diagnostic media. All these organisms were tested as streak cultures on tri-olein agar plates.

Lipolysis media. Tributyrin agar was made up as in B.S.S. 895 (1940) and was essentially a 1% emulsion of tributyrin in yeast extract, meat extract, and peptone-agar base, emulsification being accomplished in a domestic hand emulsifier ('cream' machine).

The triolein media were of four kinds:

(1) 'W.G.N.R.'; yeast extract peptone agar with 1:200,000 neutral red and 2% triolein saturated with waxolene green (I.C.I.) (Sadek (6)).

(2) 'O.B.A.B.'; yeast extract peptone agar with 1:6600 aniline blue (spirit blue) and 1% triolein saturated with the oxazone base of nile blue (Sadek (6)).

(3) 'Nile blue'; yeast extract peptone agar with 1:10,000 nile blue and 1% triolein (modified from Turner (7)).

(4) '$CuSO_4$'; yeast extract peptone agar with 1% triolein, the medium treated with saturated aqueous $CuSO_4$ after incubation (Berry (8)).

In each of the triolein media the sterile fat was added to 10 ml. agar in a test-tube fitted

with a rubber stopper and dispersed by hand-shaking (for details see Jones & Richards (4)). The cultures tested on tri-olein media were grown as surface streak plates incubated for 5 days at 30° C.

RESULTS

The effect of heat upon the tributyrinolytic count

Counts of lipolytic colonies were made of twelve samples of cream plated with tributyrin agar, before and after the two methods of heat treatment. The results, given in Table 1, show a considerable reduction of tributyrinolytic colonies after heating. The 'hold' method is clearly the more effective with a percentage reduction varying from 98·25 to 100%, while the less easily controlled 'flash' process had a reduction ranging from 97·5 to 100%

Table 1. *Colony counts of tributyrinolytic organisms in raw and pasteurized cream*

Sample no.	Raw	Colony count/ml. 'Hold' pasteurized	Colony count/ml. 'H.T.S.T.' pasteurized
1	383,000	4,000	7,000
2	425,000	4,000	10,000
3	460,000	5,000	2,000
4	363,000	5,000	9,000
5	503,000	None survived	4,000
6	1,850,000	5,000	9,000
7	459,000	8,000	10,000
8	512,000	7,000	12,000
9	495,000	4,000	10,000
10	515,000	9,000	7,000
11	348,000	4,000	7,000
12	122,000	None survived	None survived

The lipolytic organisms in raw cream

Representative colonies lipolytic on tributyrin agar were picked off for characterization and action on triolein as a test of their true lipolytic ability. The characterization was in most cases carried to species determination according to Bergey's *Manual*, but in this account the organisms are grouped for simplification in six broad categories: (1) Micrococci—Gram-positive and Gram-variable catalase-positive mass-forming cocci; (2) Achromobacteria—Gram-negative rods which do not ferment lactose and produce no water-soluble fluorescent pigment (a number of yellow pigmented *Flavobacterium* species are included here); (3) Pseudomonads—Gram-negative rods producing yellow to green fluorescent pigment soluble in agar medium; (4) Bacilli—Gram-positive or Gram-variable rods producing endospores on yeast extract agar slopes; (5) Coryneforms—Gram-positive small rods characteristically arranged in small bundles and not producing endospores; (6) Actinomycetes—tough adherent colonies which stained as long filamentous branched threads. It might be argued that this grouping over-simplifies the ecology of tributyrinolytic colonies in cream; but, together with lactic streptococci, which do not grow on tributyrin agar, and the *coli-aerogenes* bacteria, none of which attacks tributyrin, these organisms in any case make up the majority of the flora of milk and milk products. The number of organisms examined in the twelve cream samples was 504; their distribution in the various categories and their effect on tri-olein in the four media are given in Table 2.

Nile blue gives a poor performance as an indicator in that 163 of the 270 Micrococci species, eighteen of the twenty Bacilli group, and all the Coryneform rods were unable to grow in the presence of this dye. The results in the other three triolein media

Table 2. *Tributyrinolytic organisms in raw cream and their lipolytic effect on tri-olein shown by various methods*

Bacterial group	No. lipolytic on tributyrin	No. lipolytic on triolein as shown by W.G.N.R.	O.B.A.B.	$CuSO_4$	Nile blue
Micrococci	270	203	203	203	107 (163 no growth)
Achromobacteriaceae	166	74	74	74	74
Pseudomonads	34	33*	33	33	33
Bacilli	20	0	0	0	0† (18 no growth)
Coryneforms	14	0	0	0	None grew

* The remaining strain was slightly lipolytic.
† The other two strains grew slightly on nile blue.

were consistent and showed that the numbers attacking triolein were much fewer than those 'lipolytic' on tributyrin. This is a reflexion of earlier findings with tributyrin and butterfat (Jones & Richards(3)). By far the predominant bacteria were Micrococci and Pseudomonads.

Lipolytic organisms in cream after 'hold' pasteurization

Fifty-five cultures of tributyrin-positive organisms were taken from the twelve cream samples. These fell into four broad groups differing from the organisms from raw cream in that the heat-labile Gram-negative rod categories had been eliminated and two filamentous organisms of Actinomycetes made their appearance. The numbers in each group and their effect upon triolein are given in Table 3. Both the Actinomycetes were lipolytic, but Micrococci were the predominant triolein-splitting organisms. The most numerous group, aerobic sporeformers, were uniformly non-lipolytic on triolein. Fifty-three of the fifty-five cultures were unable to grow on nile-blue agar.

Table 3. *Tributyrinolytic organisms from cream after 'hold' pasteurization and their lipolytic effect on triolein shown by various methods*

Bacterial group	No. lipolytic on tributyrin	No. lipolytic on triolein as shown by W.G.N.R.	O.B.A.B.	$CuSO_4$	Nile blue
Micrococci	16	13*	13	13	None grew
Bacilli	26	0	0	0	None grew
Coryneforms	11	0	0	0	None grew
Actinomycetes	2	2	2	2	2

* One of the other three was slightly lipolytic.

Lipolytic organisms in cream after 'H.T.S.T.' pasteurization

Survivors were more numerous in this treatment and ninety-one tributyrin-splitting colonies were available for further study. They fell into the same four categories as in the 'hold' process, but here the Micrococci group were most numerous and dominated the triolein-attacking flora (Table 4). As in the previous process, none of the Bacilli or Coryneforms groups was truly lipolytic. Thirty-four of the forty-eight Micrococci and the one Actinomycete attacked triolein. Only one of the ninety-one organisms grew on nile blue.

The effect of 24 hr. storage on the flora of raw and pasteurised creams

A common procedure in butter factories and on dairy farms is to store cream at cool temperatures prior to processing. It is thus of some interest to study the change of

Table 4. *Tributyrinolytic organisms from cream after* 'H.T.S.T.' *pasteurization and their lipolytic effect upon triolein shown by various methods*

Bacterial group	No. lipolytic on tributyrin	No. lipolytic on triolein as shown by W.G.N.R.	O.B.A.B.	$CuSO_4$	Nile blue
Micrococci	48	34*	34	34	None grew
Bacilli	18	0	0	0	None grew
Coryneforms	24	0	0	0	None grew
Actinomycetes	1	1	1	1	1

* Four of the remaining strains were slightly lipolytic.

lipolytic flora in cream, both raw and heated, brought about by 24 hr. storage of the raw cream in a cool place.

The samples were similar to those previously used. Each was divided into two portions, of which one was at once examined by plating on tributyrin agar before and after heat treatment by 'hold' and 'flash' processes. The second portion was held for 24 hr. in a container in running water, at a temperature ranging between 8 and 11° C., before being similarly examined by plating on tributyrin agar raw and after heating.

Table 5 gives the colony counts on tributyrin agar of the raw and pasteurized samples of four creams, before and after storage. In the raw creams there is a marked increase in tributyrin-splitting organisms during 24 hr. storage, ranging from 8- to 13-fold. The storage period, however, seems to have no marked effect upon the organisms surviving heat treatment. Indeed, the thermoduric figures for fresh and stored creams correspond well with those in the earlier heat-treatment experiments on fresh samples.

Table 5. *Colony counts of tributyrinolytic organisms in raw and pasteurized cream before and after storage for* 24 *hr. at* 8–11° C.

Sample no.		Raw	'Hold' pasteurized	'H.T.S.T.' pasteurized
1	Before	463,000	2,000	7,000
	After	5,100,000	None	6,000
2	Before	385,000	6,000	8,000
	After	3,700,000	4,000	8,000
3	Before	1,650,000	5,000	4,000
	After	21,400,000	6,000	5,000
4	Before	522,000	None	6,000
	After	4,500,000	3,000	7,000

Lipolytic flora of raw and pasteurized cream before and after storage

The colonies picked off from tributyrin agar plates were grouped and tested as before. The results are given in Table 6. From the raw cream before storage 116 colonies were examined from the four samples and 123 after storage. The most numerous groups were Micrococci and Achromobacteria rods, and the significant result of storage was the increase of Pseudomonads and Achromobacteria and the relative decrease of Micrococci and Coryneforms. Regarding the true lipolytic nature of these organisms, as before, the significant organisms were the Micrococci, the Pseudomonads and, to a lesser degree, the *Alcaligenes* and *Achromobacter* species of the Achromobacteria. The effect of storage is to increase the lipolytic Gram-negative rods so that the relative importance of the Micrococci is lessened.

In the 'hold' process of heat treatment, from the four samples thirteen cultures were

available for study from each of the fresh and stored creams. These belonged in each case to Micrococci, Bacilli and Coryneforms groups only, and of these only the Micrococci were truly lipolytic. The same three groups were found in the 'flash'-heated cream, twenty-five colonies being examined from fresh, heated and twenty-six from stored, heated cream. The results of the earlier experiments are reflected here in that there is a greater proportion of Micrococci in the 'flash'-heated cream and thus a more active lipolytic population.

Table 6. *The lipolytic effect on tri-olein of the tributyrinolytic flora of cream before and after 24 hr. storage at* 8–11° C. (*a*) *raw*, (*b*) '*hold*' *pasteurized and* (*c*) 'H.T.S.T.' *pasteurized*

		Raw		'Hold'		'H.T.S.T.'	
Bacterial group	Action on	Before	After	Before	After	Before	After
Micrococci	Tributyrin	57	44	3	4*	13*	12
	Triolein	44	37	2	3	10	11
Bacilli	Tributyrin	5	4	4	4	6	6
	Triolein	0	0	0	0	0	0
Coryneforms	Tributyrin	8	5	6	5	6	8
	Triolein	0	0	0	0	0	0
Pseudomonads	Tributyrin	5	11	0	0	0	0
	Triolein	4	10	0	0	0	0
Achromobacteria	Tributyrin	41	59	0	0	0	0
	Triolein	14	37	0	0	0	0

* One of the strains was slightly lipolytic on triolein.

DISCUSSION

Tributyrin agar is widely used for the detection of lipolytic micro-organisms, and in Britain is the officially recommended medium for this purpose in regard to dairy products. The medium has the attraction of being fairly easy to prepare and of giving very clear-cut results, because the water-soluble products of hydrolysis leave a clear transparent zone around lipolytic colonies in an opaque medium. It is, however, quite widely appreciated that the ability of an organism to hydrolyse tributyrin in an agar emulsion does not imply that it can cause rancidity in more complex natural fats.

Jones & Richards (4) and Sadek (6) have found that there is a good agreement between lipolysis results on triolein and on the natural fats, olive oil and butter, and this has received support from the finding by Richards & Sadek (5) of a large oleic acid-like fraction in the acids recovered from rancid butter. On this evidence, in the present work we have enumerated tributyrinolytic organisms in cream and have tested their 'true' lipolytic nature on triolein agar.

It is interesting to find that of the 967 tributyrin-splitting organisms from cream tested in this work 55% were lipolytic on triolein, whilst in an examination in this laboratory, a few years ago, of 962 bacteria from various sources (mainly milk and butter)— a collection quite distinct from the present one—Jones & Richards (4) found that of the 752 which attacked tributyrin 426 (or 56%) were lipolytic on butterfat agar. The similarity of the two sets of figures on quite large collections of mainly milk, cream and butter organisms is very striking. The large proportion of true lipolytic bacteria belonging to the Micrococci group (66% in the raw creams and 94% of those in heated creams) should also be emphasized again, as it was by Richards & Jones.

The effect of heat treatment on cream is to reduce the count very considerably—by

more than 97%. The 'hold' process on the laboratory test-tube scale is, as one would expect, more effective and uniform in this reduction. Small-scale 'batch' H.T.S.T. cannot be controlled accurately, but the results obtained bear a similar relationship to the 'hold' process as do those of commercial H.T.S.T. to holder pasteurization of milk in regard to the numbers and types of organisms surviving. The reduction in total numbers of organisms must result in improved keeping quality of the cream, but it is probable that the elimination by heat treatment of the souring organisms will allow freer development of the lipolytic organisms which survive.

The effect of a day's storage in cool conditions is to allow an increase in the lipolytic flora of raw cream. This increase is largely made up of Gram-negative rods of heat labile Pseudomonads and Achromobacteria groups, and as these are killed by heat treatment and the Micrococci do not appear to increase during the storage period, the effect of heat treatment is to nullify the effects of storage as regards lipolytic flora.

All the triolein media used gave clear-cut results with the organisms tested as surface streak cultures. There is no doubt that many effective lipolysis media with indicator dyes can be used to test organisms for lipase activity, but such media are slightly more troublesome to make up than are the simpler yeast or meat extract basal media. It is, however, important to ensure that the indicator dye used has no inhibitory action on the organisms tested. Gram-positive bacteria, including the significant *Micrococcus* genus, are very susceptible. Nile blue is typical of this kind of dye and the results in this work clearly show its limitations.

SUMMARY

The effect of 'hold' and 'H.T.S.T.' heat treatment on cream was a 97–100% reduction in the tributyrinolytic flora. There was no significant difference between the two methods in this respect. The tributyrin-splitting organisms have been tested for their 'true' lipolytic effect upon triolein; of 967 organisms tested 55% attacked triolein. In raw cream the most numerous lipolytic organisms were of the Micrococci and Pseudomonads groups, whereas in heated cream the former were by far the predominant lipolytic organisms.

The result of storing cream at 8–11° C. for 24 hr. was an 8- to 13-fold increase in tributyrinolytic organisms, mostly of Gram-negative rods many of which attacked triolein. The effect of heat treatment nullified this increase and the lipolytic flora of heated cream was much the same whether or not the cream was stored before heating.

The triolein media incorporated two new indicator dye combinations which were successful but a third, nile blue, was found to be strongly inhibitory to the majority of triolein-splitting organisms.

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595. THE STEAM STRIPPING OF TAINTS FROM LIQUIDS

III. BATCH STRIPPING WITH PARTICULAR REFERENCE TO EQUILIBRIUM VALUES FROM BATCH DATA

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(With 5 Figures)

INTRODUCTION

Previous papers (3,4), Parts I and II, in this series have shown methods of computing steam consumption and taint removal for multiple contact in both co-current and counter-current forms. The third basic type of equipment which can be used for the steam stripping of taints from liquids is the batch method (1), in which a charge of liquid containing the taint is placed in a vessel then steam is blown through the liquid until a suitable degree of deodorization has been effected.

Batch methods are in common use in the deodorization of edible oils but are not used on a commercial scale for cream. Common chemical laboratory practice for analytical and experimental work involves the use of batch methods of stripping and distillation, and it is often important to be able to use laboratory results, determined with batch apparatus, in the design or operation of many types of industrial plant. Consequently one object of this paper is to show how the equilibrium curve can be derived from the results of batch operations. From the equilibrium curve, representing the concentration of taint in the steam which is in equilibrium with some concentration of taint in the liquid, the methods previously presented in Parts I and II can be used to calculate plant performance.

Steam stripping and steam distillation

The several branches of the vapour-liquid transfer operation have been given designations, admittedly often in an arbitrary manner. The process of passing steam through cream, or through water containing a volatile solute, has been termed stripping in the series, in accordance with chemical engineering practice. The same process has been termed steam distillation by some workers who have followed the terminology of chemical laboratory practice, but the present author has restricted this latter designation to a process in which steam is supplied to a non-aqueous liquid for the purpose of distillation at a lower temperature. The actual nature of the steam deodorization process for an aqueous liquid is distillation by means of open steam.

Batch operation with constant liquid weight—open steam

Fig. 1 shows in diagrammatic form a batch system of deodorization in which it is assumed that no heat need be supplied from the steam for purposes of heating the charge or supplying heat losses. Since the weight of taint is very small, the weight of the liquid in the vessel is constant for all practical purposes. As the total weight of steam passing

through the system increases with increasing time, the residual taint concentration decreases in a manner represented by the curves in the bottom diagrams of Figs. 2 and 3. The mathematical equations relating residual taint concentration and total steam used are derived in the appendix, where it is shown that for a solute obeying Henry's law

$$\frac{x_0}{x_1} = e^{-EF},$$

where E is the efficiency of contact between the steam and the liquid expressed as the ratio of taint concentration in the steam to equilibrium concentration in the steam, F is the stripping factor mv/L and m is the equilibrium constant, v the total pounds of steam, and L the pounds of liquid.

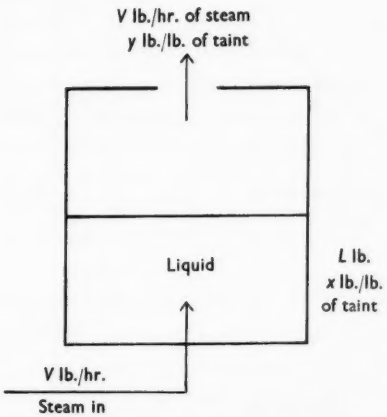

Fig. 1. Diagram of a simple batch deodorization process in which steam is bubbled through a liquid containing a volatile taint.

Where the contact conditions are suitable the value of E can reach the maximum value of 1·0, in which case the equation becomes $x_0/x_1 = e^{-F}$ which is identical with the equation already derived in Part I for a system of an infinite number of contacts. The batch system thus gives the same degree of taint removal as a co-current infinite contact system and represents the limiting value of taint removal which can be achieved by multiple-contact methods (5).

The general case where the equilibrium characteristic is not linear can be solved by graphical integration. The appendix shows that the total pounds of steam used per pound of liquid in the batch is equal to

$$\frac{v}{L} = -\frac{1}{E}\int_{x_1}^{x_0} \frac{dx}{y_e},$$

provided no steam is generated within the charge and no condensation of the injected steam takes place. If the equilibrium relation between the taint in the steam and the taint in the liquid is known, the equation can be solved when E is known or assumed.

Fig. 2 illustrates the method of graphically computing the curve of steam consumption from any equilibrium curve for a case where intimate steam–liquid contact is obtained ($E = 1$). The equilibrium curve is shown in the top section of the diagram. The middle section is derived from the y_e versus x curve in the top section by plotting values of $1/y_e$ for

various values of x. The steam used to reduce the solute concentration from 5 to 3·5 p.p.m. can be obtained by measuring the area under the reciprocal curve between the limits $x=5$ and $x=3{\cdot}5$. This area is the cross-hatched portion under the line OB. The ordinate at O is 0·006 and the ordinate at B is 0·01, hence the average ordinate over a base-line of 1·5 is 0·008 and the enclosed area is very nearly $1{\cdot}5\times0{\cdot}008$, equal to 0·012, which gives the total steam which would be used. Point C in the bottom section represents the value 0·012 just calculated. To obtain the amount of steam used to reduce the concentration to 3 p.p.m., the area under OE must be measured. Thus the ordinate at B is 0·01 as before, and

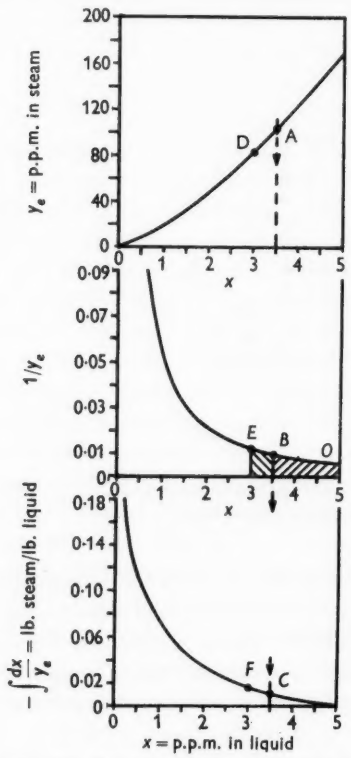

Fig. 2. The method of obtaining the amount of steam used to effect the required final taint concentration for the constant-weight method.

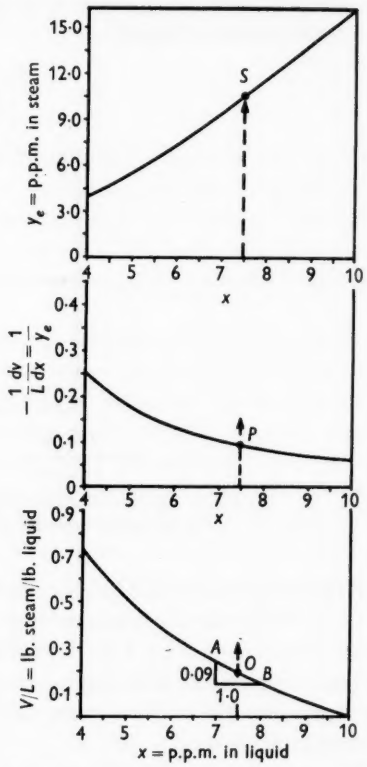

Fig. 3. The method of obtaining the equilibrium relation y_e against x from the steam consumption curve for the case of constant weight.

the average ordinate is 0·011 between $x=3{\cdot}5$ and $x=3{\cdot}0$. The area under the curve BE is therefore $0{\cdot}5\times0{\cdot}011$ or 0·0055, and the total area under OE is $0{\cdot}012+0{\cdot}0055$ or 0·0175, which is shown plotted as point F in the lower diagram. Proceeding in this fashion the complete range of values can be computed and the curve drawn as in the bottom section. Had the value of E not been unity but say 0·5, then all the steam values as obtained above would have to be divided by 0·5 in order to get the correct result.

In the above method, the area under the reciprocal curve was obtained by assuming that the incremental lengths such as OB or BE were straight, and in order to get a reason-

ably accurate result, the points such as B and E must be spaced along the curve to keep the incremental lengths approximately linear. Instead of the above method, Simpson's rule can be used to give slightly greater accuracy, or the area can be measured with a planimeter.

The reverse procedure of deriving the equilibrium curve from the curve representing the relation between the residual solute and the steam used is a graphical differentiation based on the equations derived in the appendix. Fig. 3 illustrates the graphical method based on the equation

$$\frac{1}{y_e} = -\frac{E}{L}\frac{dv}{dx}.$$

In Fig. 3, E is taken as unity. The points A and B in the bottom section are taken sufficiently close together so that the portion of the curve between them is approximately linear. The small triangle can be used to measure the slope at a point midway between A and B, the slope being equal to $1/L.dv/dx$. The actual value of the slope is $-0{\cdot}090 \div 1{\cdot}0$, equal to $-0{\cdot}090$, hence the value of $1/y_e$ at $x = 7{\cdot}5$ is $0{\cdot}090$ and y_e is $11{\cdot}1$ p.p.m., as shown by point S in the top section. The complete equilibrium curve can be constructed by taking other points similar to A and B. The equilibrium curve obtained in Fig. 3 is approximately that for acetoin in water (see Parts I and II). It is not necessary to draw the intermediate curve shown as the middle section of Fig. 3, but it is included in the diagram in order to show the link between the original and derived curves.

Batch operation with varying liquid weight-generated steam

Data sometimes can be obtained from the results of laboratory experiments in which an aqueous liquid is deodorized by boiling, the steam being produced by evaporation of part of the liquid. Because the weight of liquid constantly decreases, the methods given for open steam must be changed to allow for the altered conditions.

The basic equation for the use of generated steam is derived in case II of the appendix as

$$\frac{\text{Total steam generated}}{\text{Initial liquid weight}} = \frac{v}{L} = 1 - e^{R},$$

where

$$R = \int_{x_1}^{x_0} \frac{dx}{Ey_e - x}.$$

If the equilibrium curve y_e versus x is available and the efficiency of contact E is known, then in order to find the amount of steam required to produce some final concentration of taint the following steps are made:

(1) Draw a curve of $1/(Ey_e - x)$ versus x.

(2) Integrate this curve from the initial value x_1 to the required final value x_0 and so obtain values of R corresponding to values of x.

(3) Calculate values of v/L from the expression $1 - e^{R}$.

The procedure for obtaining the equilibrium relationship from the curve expressing steam used to produce some remaining concentration in the liquid, is the reverse of (1) to (3) above:

(*a*) From the curve of v/L versus x a curve of $\log_e \dfrac{1}{1 - v/L}$ versus x is drawn.

(*b*) This derived curve is differentiated to produce the relation $\dfrac{1}{Ey_e - x}$ versus x.

(*c*) If E is known or assumed, the values of y_e corresponding to x can be obtained since

$$Ey_e = \frac{1}{1/(Ey_e - x)} + x.$$

The methods are illustrated in Figs. 4 and 5 in which Fig. 4 shows the derivation of the steam line from the equilibrium curve for diacetyl in water; and Fig. 5 shows the derivation of the equilibrium curve from the steam line for acetoin in water.

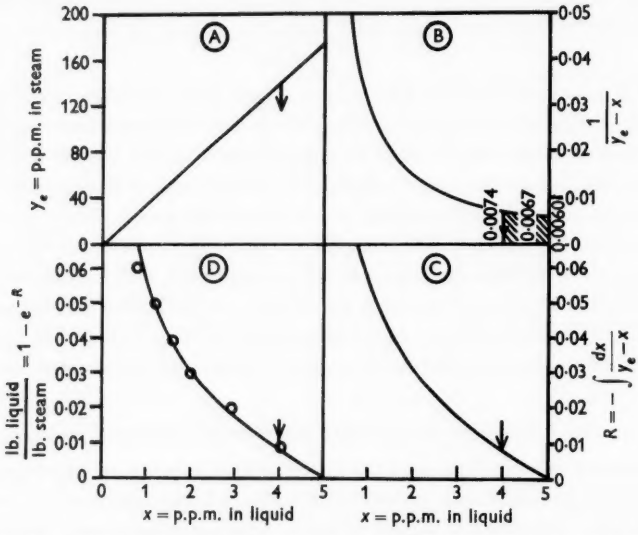

Fig. 4. The method of obtaining the amount of steam used to attain the required final taint concentration for the case where the liquid is boiled and weight decreases. Curves are for diacetyl in water and experimental values are plotted for comparison with the calculated curve.

Fig. 4A shows the diacetyl equilibrium curve and 4B shows the derived curve $\frac{1}{y_e - x}$ (where E is taken as unity) and the integration of this curve in a manner already described for Fig. 2. For example, at the liquid concentration $x=4$ the area under the curve is $0{\cdot}0067 \times 1{\cdot}0$. Other points obtained in this way complete the curve shown in Fig. 4C, allowance being made for the change in sign of the integral. The curve in Fig. 4D is $v/L = 1 - e^{-R}$ (where R is taken as positive) or $v/L = 1 - (0{\cdot}368)^R$. The plotted points are experimental results obtained by boiling 500 ml. of water-diacetyl solution in a 1000 ml. flask, and condensing and measuring the weight of steam formed. The taint determination was done by a method due to McDowall (see (4), Part I), and the rate of steam evolution was 2 ml. per minute. There is good agreement between the calculated and experimental results.

Fig. 5 shows the method of deriving the equilibrium curve from the experimentally determined steam line in Fig. 5D. Taking the point 8 p.p.m. and 0·32 lb. steam (per pound of original liquid weight) the corresponding point in Fig. 5C is

$$\log_e 1/(1 - 0{\cdot}32) = \log_e 1{\cdot}45 = 0{\cdot}371,$$

and the slope is $-(0{\cdot}49 - 0{\cdot}25)/1$ or $-0{\cdot}24$ and is plotted in Fig. 5B as the negative differential. Since $1/(y_e - x)$ must equal 0·24 in this example, $y_e = 1/0{\cdot}24 + 8$ or 12·16

shown plotted in Fig. 5A. Other points on the equilibrium curve can similarly be plotted. The graphical procedure illustrates clearly the method, but in practice a tabular method is all that is required.

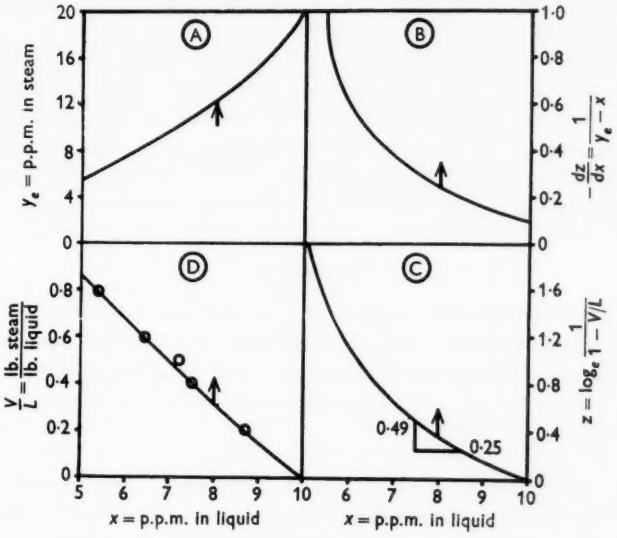

Fig. 5. The method of obtaining the equilibrium relation y_e against x from the steam consumption curve for the case where the liquid is boiled and the weight decreases. Curves are for acetoin in water.

DISCUSSION

In simple flask apparatus there can be errors in experimental results due to several reasons. Small amounts of reflux or run-down are always present, for example; and for the constant weight method where steam is bubbled through a liquid, one cannot always assume complete saturation of the steam ($E = 1$) unless precautions are taken to ensure small steam-bubble diameters or sufficient height of liquid above the steam bubble inlet(1,2). Furthermore, in spite of precautions, steam may be used to keep the flask and its contents at the requisite temperature. An alternative method is to boil the liquid directly, but allowing constant water make-up to keep the weight of liquid constant. It would seem that by the nature of the formation and growth of the steam bubbles that complete saturation could be expected. The heat from the bunsen burner should also help to reduce the reflux problem. The advantages of steam generation within the flask can be kept by the variable weight method given above with the advantage that the apparatus can be kept to its simplest possible form.

The equilibrium curve shown in box A of Fig. 4 was derived by McDowall for a commercial sample of diacetyl used in a comparative test of two types of deodorizing apparatus. The equilibrium coefficient of 35 is about 10% lower than the coefficient for pure diacetyl.* The acetoin used in the experiment for which the results in Fig. 5 are given was commercial acetoin used in similar comparative trials. Box A shows that the true equilibrium coefficient 1·29* is not reached until the higher volatile portion is distilled. The initial coefficient

* Private communication from F. H. McDowall.

is 2·0, hence if the impurity is diacetyl having an equilibrium coefficient of 39·3 the increase could be accounted for by the presence of 1·85% of diacetyl. In many cases of comparative tests of machinery, purity of the reference substance is not important, and the presence of some impurities may even be desirable if the resulting 'equilibrium curve' more closely represents conditions in practice.

The use of properly constructed stills for the determination of equilibrium relationships will give more accurate figures than the simple flask apparatus dealt with in this analysis, but in cases where such apparatus is not available or where results of simple flask distillations are given in the literature the methods set out in this paper can be used to evaluate the equilibrium curve of a substance in solution. This derived equilibrium curve can then be used to compute the efficacy of deodorizing plant in removing the substance from solution.

SUMMARY

Methods are given for calculating the degree of taint removal from systems in which steam is bubbled through the liquid, and systems in which the liquid is boiled. The reverse procedure of obtaining equilibrium curve data from results of batch experiments is also shown. Examples are given for experimental values for removal of diacetyl and acetoin from solution by boiling.

The author would like to thank Dr F. H. McDowall, Chief Chemist of the Dairy Research Institute (N.Z.), for helpful discussion and criticism; and for facilities in connexion with analyses of diacetyl and acetoin samples. The author wishes also to express appreciation to Mr E. R. Elley of the Chemistry Department for his painstaking care in the analysis of samples.

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NOMENCLATURE

e 2·718, $1/e = 0{\cdot}368$.
E Fractional efficiency of contact between the steam and the liquid in terms of ratio of taint concentration of steam to its equilibrium values. Dimensionless.
F The stripping factor, mv/L. Dimensionless.
L Quantity of liquid initially in the batch. Lb.
m Equilibrium constant defined by $y = mx$.
R Value of integral $\int_{x_1}^{x_0} \frac{dx}{Ey_e - x}$. Dimensionless.
v Total quantity of steam used. Lb.
V Rate of steam production. Lb./hr.
x Instantaneous concentration of taint in liquid. Lb./lb.
x_1, x_0 Initial and final concentrations of taint in liquid. Lb./lb.
y Instantaneous concentration of taint in steam. Lb./lb.
y_e Concentration of taint in steam in equilibrium with x.

APPENDIX

Steam Stripping by the Batch Process

Case I. Open steam and constant liquid weight

The conditions are represented in Fig. 1, where a liquid initially of L lb. and at the saturation temperature of the steam contains x_1 lb./lb. of taint. Steam at a rate of V lb./hr. is passed through the liquid, and at any instant the concentration of taint in the outgoing steam is y lb./lb.

Since there is no change in the weight of steam passing through the system, the liquid weight is constant and the following material balance can be set up:

Taint carried away by steam in time dt = taint lost by liquid,

$$Vy\,dt = -L\,dx.$$

If the final taint concentration is x_0, then

$$-\int_{x_1}^{x_0}\frac{dx}{y} = \frac{V}{L}\int_0^t dt = \frac{Vt}{L} = \frac{v}{L},$$

where v is the total steam used.

If the relation between y and x is known the left-hand integral can be evaluated. In general $y = Ey_e$, where y_e is the equilibrium value corresponding to x and E is the fractional efficiency of contact. Hence

$$\frac{v}{L} = \frac{\text{lb. of steam}}{\text{lb. of liquid}} = -\frac{1}{E}\int_{x_1}^{x_0}\frac{dx}{y_e}.$$

If a linear relation exists between y_e and x such that when m is constant for $y_e = mx$, then

$$\frac{v}{L} = -\frac{1}{mE}\int_{x_1}^{x_0}\frac{dx}{x} = -\frac{1}{mE}\log_e\frac{x_0}{x_1}$$

or

$$\frac{x_0}{x_1} = e^{-E}\frac{mv}{L} = e^{-EF},$$

where the stripping factor $F = mv/L$.

The reverse process of determining the equilibrium relationships from the batch stripping curve is as follows:

$$\frac{1}{y} = -\frac{V}{L}\frac{dt}{dx} = -\frac{1}{L}\frac{dv}{dx} \quad \text{or} \quad \frac{1}{y_e} = -\frac{E}{L}\frac{dv}{dx}$$

If the curve of v/L against x is available, differentiation with respect to x will give the values for $1/y$, or $1/y_e$ if E is known.

Case II. Generated steam and variable liquid weight

In this case an aqueous liquid is boiled by the application of heat so that the weight of liquid remaining is constantly diminishing.

If at some instant there are l lb. of liquid containing a taint of x lb./lb., the total weight of taint at any instant is lx lb. After a small increase of time the new weight of liquid will be $(l-dl)$ lb. and the taint concentration $(x-dx)$ lb./lb. The new weight of taint remaining is $(l-dl)\,(x-dx)$ lb., and equating the loss of taint in the liquid to that leaving with the steam (total weight dl lb.) with taint concentration y:

$$ydl = lx - (l-dl)\,(x-dx) \quad \text{or} \quad \frac{dl}{l} = \frac{dx}{y-x}.$$

If the initial weight of liquid is L lb. and initial taint x_1 lb./lb., and v lb. of steam are formed, then

$$\int_{L}^{L-v} \frac{dl}{l} = \int_{x_1}^{x_0} \frac{dx}{Ey_e - x},$$

$$\log_e \frac{L-v}{L} = \int_{x_1}^{x_0} \frac{dx}{Ey_e - x} = R,$$

and is the well-known Raleigh equation, from which

$$\frac{v}{L} = \frac{\text{lb. steam}}{\text{Original liquid weight}} = 1 - e^R.$$

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596. STEAM DISTILLATION OF TAINTS FROM CREAM

I. THEORETICAL CONSIDERATIONS AND PROPERTIES OF THE REFERENCE SUBSTANCES, DIACETYL AND ACETOIN

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(With 7 Figures)

Cream may contain off-flavours: (*a*) derived from weeds or feeds as taints transmitted via the blood stream to the milk during its secretion by the cow; or (*b*) produced by the cow under disturbed conditions of metabolism, e.g. digestion upsets, and passed on to the milk via the blood stream; or (*c*) absorbed from the atmosphere or from utensils during the interval between production of milk or cream at the farm and delivery at the factory; or (*d*) formed by bacterial action during this same period. These taints if left in the cream may render the resultant butter of low grade or definitely unpalatable.

The taints present in the cream may be removable in part or in whole in one or other of several ways:

(i) *By washing*, i.e. by mixing the cream with untainted skim milk, or with untainted skim milk diluted with water, or with water, and reseparating.* The washing method with *skim milk* is applied in commercial practice to some extent, e.g. in countries where neutralization with alkali is not permitted; but it is not suited for use in large butter factories owing to the difficulty of obtaining adequate quantities of untainted skim milk and to the amount of labour, time and equipment employed. Washing with *water* will remove many types of taint, but it gives a 'washed cream' flavour to the butter. At some butter factories segregated poor-quality cream is mixed with cream-can steamings (which contain much condensate) and reseparated, for improvement of the cream and butter flavour ((3), pp. 157, 371).

(ii) *By aeration*, i.e. by use of air as a carrier vapour into which the tainting substances can diffuse. This system is applied on open-surface coolers and in open cream-pasteurizers. Since the vapour pressure of the tainting substance increases with increase in temperature of the cream, aeration of the milk or cream at a high temperature is more effective than aeration at a low temperature. Aeration at a high temperature has the further advantage that the air as it is heated takes up water vapour to the appropriate saturation pressure, and this water vapour as it leaves the cream takes with it a load of the tainting substances. Hence there is appreciable taint removal in a beater-type flash-pasteurizer, especially if a fan is used to draw air over the surface of the moving cream, thus increasing the proportion of entraining agents, both air and water vapour, passing over or leaving the surface of the cream. Similarly, there is a greater deodorization at the top of an open-surface

* Since some tainting substances are not very water-soluble, use of liquid paraffin as an extracting agent has been proposed by Macdonald & Crawford(1). This method, however, has serious practical disadvantages—the manipulations involved are time-consuming, and there is difficulty in removing the liquid paraffin from the cream. McDowall(2) has tried the use of paraffin wax, but even this could not be easily removed from the cream.

cooler, where the cream temperature is high, than at the bottom, where the cream temperature is low.

(iii) *By application of vacuum.* The widely held belief that tainting materials can be removed by application of reduced pressure is valid, with aqueous solutions,* only for gaseous taints or for tainting substances of low boiling-point, present in appreciable proportion. Without the use of an entraining agent of some kind the application of a vacuum does not result in the removal of significant quantities of tainting substances when they are present in such low concentrations as is usual in commercial cream (see also (3), pp. 334–8, 372; and (3a)).

(iv) *By distillation with steam.* Steam is a more convenient carrier vapour than air because: (*a*) it facilitates control of temperature of the cream, both for pasteurization and during the deodorization process; (*b*) it is readily available in quantity as required, and by means of a suitable water condenser it can be drawn through the deodorization equipment rapidly and cheaply; and (*c*) some substances, and this probably includes some of the tainting substances, are very 'steam-volatile'.

When a vacuum is applied to hot cream, flash boiling occurs to the extent required for lowering of the cream temperature by latent heat absorption until the vapour pressure of the cream corresponds to the reduced pressure obtaining. This steam serves as a carrier vapour for tainting substances, and under these conditions application of a vacuum does lead to some deodorization of the cream; but the quantity of flash steam is limited to that required to provide the appropriate lowering of temperature, and therefore the extent of deodorization possible is limited in a similar way.†

Equipment for the deodorization of cream for butter-making, and of milk for the liquid milk trade, by use of steam as an entraining agent has been in use for more than 30 years, but there has not hitherto been any quantitative study of the effectiveness of the deodorization obtained under the various conditions of operation possible with the equipment available.

FACTORS CONTROLLING DISTILLATION OF TAINTING SUBSTANCES WITH STEAM

Most tainting substances are present in milk and cream in very small proportion. They are likely to be both fat-soluble and slightly water-soluble. Hence in cream they will be dissolved partly in the serum and partly in the fat. For purposes of simplicity in the present discussion, however, it is assumed at the outset that the taint is present in solution in water, i.e. in a single-phase system.

When steam is in contact, in a closed vessel in the absence of air, with water containing a volatile tainting substance in solution, the substance exerts a vapour pressure the magnitude of which varies with variation in the concentration of substance in the solution. If the tainting substances were present in large proportion, the relation between the vapour pressure of the substance and the vapour pressure of water for ideal mixtures would be given by Raoult's law,

$$p_t = p_t^0 \times x_t \qquad (1)$$

* In aqueous solutions the temperature of steam distillation is controlled by the pressure applied. In the refining of oils, where the temperature of the oil can be maintained at will above the boiling point of water, the most effective deodorization is obtained under high vacuum conditions.

† Apparatus depending on flash evaporation of water from cream to supply steam as the carrier vapour for deodorization purposes has been in use commercially, e.g. the Murray automatic deodorizer (N.Z. Patent no. 50,694 (1923)) and the Bell 'Volatilizer'.

where p_t is the partial pressure of the tainting substance T, p_t^0 is the vapour pressure of the pure substance T at the temperature obtaining, and x_t is the mole fraction of substance T in the solution. Similarly

$$p_s = p_s^0 (1 - x_t), \tag{2}$$

where p_s = the partial pressure of the water and p_s^0 is the vapour pressure of pure water at the temperature obtaining. Hence, combining equations (1) and (2),

$$\frac{p_t}{p_s} = \frac{p_t^0}{p_s^0} \times \frac{x_t}{1 - x_t}, \tag{3}$$

or, since p_t^0/p_s^0 is constant for any one set of conditions,

$$\frac{p_t}{p_s} = K_1 \times \frac{x_t}{1 - x_t}, \tag{4}$$

i.e. for ideal mixtures the partial pressures of the tainting substance and water would be proportional to the molecular concentrations of the tainting substance and water in the solution.

The relative weights of the tainting substance (W_t) and water (W_s) in the vapour are given by the equation

$$\frac{W_t}{W_s} = \frac{M_t \times p_t}{M_s \times p_s} \tag{5}$$

$$= \frac{M_t}{M_s} \times K_1 \times \frac{x_t}{1 - x_t}, \tag{6}$$

where M_t and M_s are the molecular weights of the tainting substance and water respectively.

In equation (6) M_t/M_s is constant. Hence the equation may be written as

$$\frac{W_t}{W_s} = K_2 \times \frac{x_t}{1 - x_t}. \tag{7}$$

Raoult's law applies exactly only to a small proportion of binary mixtures, the so-called 'ideal' mixtures, and in dilute solutions it generally applies only to the *solvent*.

In equation (7) where, as for tainting substances in cream, the solution is very dilute, $1 - x_t$ is very large in comparison with x_t and may be taken as constant. Equation (7) then becomes

$$\frac{W_t}{W_s} = K_3 \times x_t, \tag{8}$$

or

$$C_V^t = K_4 \times C_L^t, \tag{9}$$

where C_V^t and C_L^t are the weight concentrations of the tainting substance in the vapour and liquid respectively. Equations (8) and (9) signify that at equilibrium the concentration of taint in the vapour is directly proportional to the concentration in the liquid. These equations are variants of Henry's law which applies for the *solute* in a dilute solution. The term K_4 in equation (9) is the 'distillation constant' as computed by Virtanen & Pulkki (4) for a number of substances from the semi-distillation values in dilute solution.

Concurrent and counter-current flow of steam and cream

It is clear that steam will be used most effectively when it is loaded to the maximum with tainting substances as it passes to the condenser, i.e. when W_t/W_s in equation (7) is at a maximum. It follows further from equation (9) that the maximum possible loading of

the steam is that obtainable when the steam leaving the system is in contact and in equilibrium with the fresh undeodorized cream entering the system, i.e. when the partial pressure p_t of the taint in the cream is at a maximum (equation (1)), or when the concentration of taint in the cream is at a maximum (equation (9)).

In *concurrent* flow of steam and cream the steam will take up taint from the cream until, if conditions are suitable, equilibrium is reached, at which stage the concentration of tainting substance in the cream will be lowered to an extent corresponding with the amount that has passed into the steam.

In *counter-current* flow of steam and cream the steam meets a gradually increasing taint concentration in the cream until, just before it passes to the condenser, it is in contact with the raw cream containing the full concentration of taint. There are, however, major difficulties in the practical arrangement of contact of steam and cream in such a way as to give a continued approximation to equilibrium conditions as the steam passes counter-current to the cream. In laboratory and commercial practice in the fractional distillation of liquids and in the refining of oils, steam and other vapours are passed in counter-current flow against liquids, in bubble-cap and other types of fractionating columns. But because of the pronounced tendency of cream to form foam and because of the sensitiveness of cream to the action of prolonged heat treatment (which produces 'cooked' or 'stewed' flavours in the butter), it is necessary carefully to limit the time and temperature of heating of every portion of the cream; and this is not possible in any form of bubble-cap column. For deodorization of cream by the counterflow method, therefore, the cream is passed in spray or film form against the current of steam. In this type of equipment the conditions of contact of cream and steam are such that full equilibrium of the tainting substances as between steam and cream is not attained in any portion of the column, and the steam at the outlet does not carry the full theoretically possible load of tainting substances (see also (3), p. 353 and (5), p. 370). The extent to which equilibrium is approached in counter-current flow can be expected to vary with a number of factors:

(*a*) *With speed of passage of the steam past the cream*, i.e. time of contact. The lower the pressure the greater the steam volume and steam velocity and therefore the shorter the time of contact. Hence other things being equal counter-current deodorization equipment will be less effective at reduced pressures than at or near atmospheric pressure.

(*b*) *With the properties of the tainting substance itself.* Substances with a low equilibrium coefficient will require a smaller transfer from the liquid to the vapour than substances with a high equilibrium coefficient. With the former substances there is a smaller retardation in transfer, because of the lesser amount of diffusion required within the droplet and also because the steam is in contact with liquid of not widely changing content of tainting or reference substance during the whole period of its passage through the counter-current deodorization equipment. A closer approach to the equilibrium concentration of the substance in the outgoing steam against that in the incoming undeodorized cream can therefore be expected than for substances with a high equilibrium coefficient. (Results confirming this will be presented in Part III of this series.)

(*c*) *With the cream-droplet size.* Small droplets present a larger surface per unit volume for diffusion of tainting substance across the liquid–vapour interface, and also the diffusion path of the tainting substance to this interface is shorter. If there are appreciable convection currents within the droplet, this liquid-diffusion factor will be of lesser importance than if the cream within the droplet is comparatively static.

Effect of two-phase nature of cream on cream deodorization

Cream, as an emulsion of fat in skim milk, is a two-phase system. Most of the tainting substances, as stated above, can be expected to be more soluble in the fat than in the milk serum, whereas the final distillation of the taint can be expected to occur from the continuous phase, i.e. from the cream serum. In the cream as received at the factory the taint will be distributed in accordance with the solubility partition coefficients of the tainting substances as between butterfat and milk serum at the prevailing temperature. Deodorization of the cream, however, takes place at relatively high temperatures, at which the partition coefficients may be different from those at normal room temperatures (e.g. see results for diacetyl in Table 2). Hence as cream enters a deodorization unit and is heated to full temperature there may be a change in the relative concentrations of the tainting substances in the fat and the serum. As soon as steam comes into contact with the cream there will be some distillation of tainting substance from the aqueous portion of the cream, thus disturbing the partition equilibrium in the direction that diffusion of the taints from the fat to the serum will occur. This process will go on continuously as taint is removed from the cream into the steam. Just how much lag in taint removal the necessity for continuous re-establishment of partition equilibrium as between the fat and the serum will cause is not known. It could conceivably be affected by the following factors: (*a*) size of the droplet of cream in contact with the steam; (*b*) amount of convection occurring within the droplet; (*c*) size of the fat globules; (*d*) amount of convection occurring within the fat globules; (*e*) the possible barrier effect of the fat-globule adsorption layer against rapid passage of the tainting substance from the fat to the serum; and (*f*) the properties of the tainting substances, e.g. (i) the steam volatility—volatile tainting substances will be quickly removed from the aqueous portion of the droplet and so will set up a greater degree of imbalance in the partition-equilibrium conditions—and (ii) the nature of the partition equilibrium, i.e. whether the substance is more soluble or less soluble in the fat than in the serum (or whether the partition coefficient $C_{\text{fat}}/C_{\text{serum}}$ is greater or less than 1). There may also be direct loss of tainting substance from the fat globules to the steam, thus bypassing the necessity for diffusion from fat globules to cream serum before distillation into the steam takes place. As can be shown thermodynamically, the vapour pressure of the substance above the fat is the same as that above the serum when the system is at equilibrium, irrespective of the nature of the fat-serum partition equilibrium for the tainting substance.

In commercial cream treatment there will be wide variations in the conditions of deodorization applied—variations in cream-flow rate, in steam-flow rate, and in conditions of contact of steam with cream—and there certainly is a wide variation in the nature and concentration of the tainting substances to be removed. The mathematical basis for the transfer of tainting substances from cream to steam has been studied by Scott (5). While such a study serves an essential purpose for the understanding of the principles of operation and for the designing of new types of cream-deodorizing equipment, account cannot be taken of all the range of variations in conditions that may occur in commercial operation of the equipment. In the final analysis, therefore, the full assessment of the efficiency of a unit will depend on practical trials under normal conditions of operation. The present and subsequent papers in this series supply the results of some investigations on the

factors affecting the rate of steam distillation and the efficiency of steam utilization in some commercial cream-deodorizing equipment.

Volatility of tainting substances in relation to rate of removal from cream

There are three aspects to the study of the volatility of a tainting substance in relation to its rate of removal. These are: (*a*) the relative volatility of the pure substance as compared with that of water; (*b*) the effect of change in pressure on the relative volatility of the pure substance; and (*c*) the volatility of the substance in dilute solution in presence of steam.

(*a*) *Relative volatility of the pure substance.* This is indicated at any one temperature by the vapour pressure of the substance, or at any one pressure by the boiling-point of the substance at that pressure. Hence boiling-points of substances at atmospheric pressure are an index of their relative volatility. When two substances are mixed, the presence of one substance affects the volatility of the other. The magnitude of this effect is expressed, for ideal mixtures, by Raoult's law (equation (1)). Since substances distil in proportion to their partial pressures, it follows from equation (3) that for ideal mixtures at any one relative concentration in the liquid the molecular proportion of substances in the vapour is directly related to the relative vapour pressures of the pure substances at the temperature obtaining. Since the vapour-pressure/temperature curves for normal substances are of a somewhat similar pattern it follows that, in general, with 'ideal' binary mixtures the higher the relative boiling-point the lower the relative volatility of the substance in the binary mixture (but see also next two sections).

(*b*) *Effect of pressure on the relative volatility of the pure substance.* Most cream-deodorization devices operate under partial vacuum or under pressures only slightly above atmospheric pressure. It is of interest, therefore, to consider what is the effect of change in pressure within this range of variation on the relative vapour pressures of the volatile substances and water. Sidgwick(6) has stated that 'if two vapour pressure curves are compared it will generally be found that as the temperature rises the ratio of the vapour pressures of any two substances approaches unity and that the efficiency of steam distillation will be greater the higher the temperature or the greater the pressure at which the steam distillation is carried out'. The effect of change in actual pressure on the ratio of the vapour pressures of a number of organic substances to the vapour pressure of water is shown in Fig. 1. The basic data were taken at random from the extensive series of vapour pressure values published by Stull(7). It will be seen that there is a considerable variation in the effect of pressure (or temperature) on the vapour-pressure ratios. For substances of boiling-point lower than that of water (Fig. 1 C) there is generally a reduction in the vapour-pressure ratio with increase in pressure or temperature. In other words, substances with boiling-point below that of water could, if the system were ideal, be expected to be more readily removed from cream at low temperatures, i.e. with application of partial vacuum. Because of the steepness of the slope of the curves at low pressures, the effect could be expected to be greater at pressures up to 200 mm. than over the pressure range 200–760 mm. mercury. In cream-deodorization equipment, such as the Vacreator,* the major deodorization is applied at pressures ranging from 380 to 760 mm. (vacuum of 15–30 in. of mercury). Over this range of pressure for low-boiling substances change in the

* Vacreator is the registered trade mark of the Murray Vacuum Cream-pasteurizer manufactured by Murray Deodorizers Ltd, Auckland, New Zealand.

substance/water vapour-pressure ratio is relatively small, but for the substances cited in Fig. 1 C, except for ethanol, it is still significant. For some of the substances with boiling-points at atmospheric pressure not widely different from that of water (Fig. 1 B) the vapour-pressure ratio rises as would be expected from the Sidgwick rule quoted above, but for many of them it falls with rise in pressure, i.e. it recedes from unity rather than approaching unity. For substances of high boiling-point (Fig. 1 A) there is a rise in the ratio with rise in pressure and the Sidgwick rule applies, but the slopes of the curves, over the range of pressures commonly employed in cream deodorization equipment, are very variable.

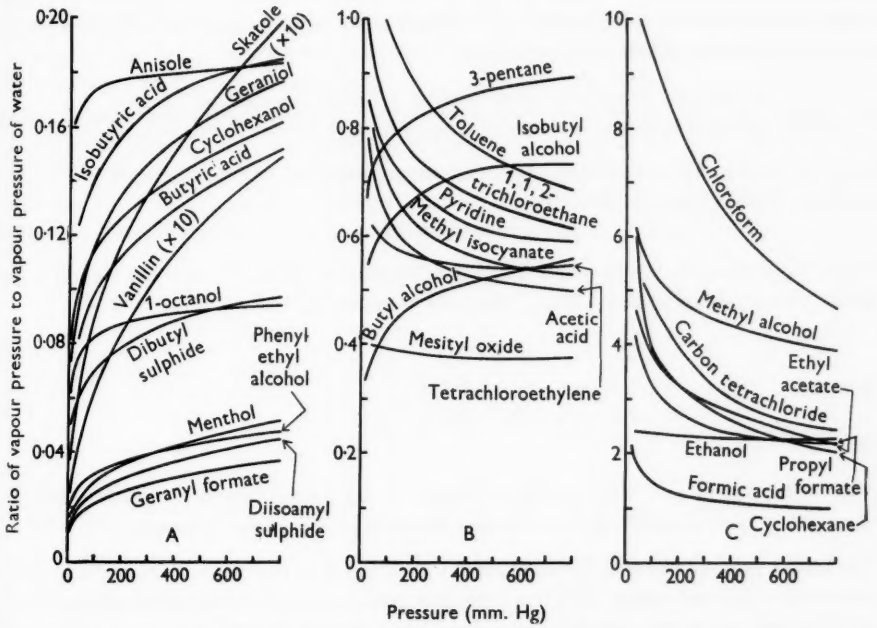

Fig. 1. Effect of change in pressure on ratio of vapour pressures of organic substances to vapour pressure of water over the pressure range 0–800 mm. of mercury

If it be assumed that tainting substances in cream are of as diverse properties as for the substances represented by the curves in Fig. 1, we may conclude that for ideal solutions lowering the temperature and pressure, i.e. increasing the 'vacuum applied': (i) would facilitate the removal of low-boiling substances; (ii) may facilitate or may retard the removal of substances with boiling point close to that of water; and (iii) would definitely retard the removal of high-boiling substances—these latter substances should be more readily removable by steam distillation at high pressure (see, however, next section).

(c) *Volatility of the substance in dilute solution.* Most binary mixtures do not comply with the ideal relationship expressed by Raoult's law, even when the proportion of each component present is considerable. Other factors, such as chemical affinities and antipathies, come into play, causing one substance to influence the properties of the other. In dilute solution the extent of variation from the ideal can be very great for the substance

present in small proportion, and especially when, as in cream, water is the main constituent of the binary mixture. Some substances have a very much higher vapour pressure in the presence of water than would be expected from the application of Raoult's law. Hence the relative volatility of a substance as indicated by its boiling-point under atmospheric pressure, or by its vapour pressure in the pure state at any chosen temperature, cannot be taken as a reliable index of the volatility of that substance in steam. Thus formic acid, b.p. 101°, is less volatile with steam than acetic acid, b.p. 118°, which is less volatile with steam than propionic acid, b.p. 141°, which in its turn is less volatile with steam than butyric acid, b.p. 163° (compare also the series methanol, ethanol, propanol and butanol). The degree of divergence of the vapour pressure of a substance (T) in a binary mixture from what would be expected if the mixture followed Raoult's law is represented by the *activity coefficient*, γ, i.e.

$$\gamma = \frac{\text{actual partial pressure of substance } T \text{ above the solution}}{\text{expected partial pressure if solution of substance } T \text{ obeyed Raoult's law}} = \frac{p'_t}{p_t}, \quad (10)$$

where p'_t and p_t are actual and ideal vapour pressures of the substance T above the solution. From equation (1) above

$$p_t = p_t^0 \times x_t. \quad (11)$$

And if x'_t is the mole fraction of a solution of substance which if it obeyed Raoult's law would give a partial pressure of p'_t, then, by equation (1),

$$p'_t = p_t^0 \times x'_t. \quad (12)$$

Hence

$$\gamma = \frac{p_t^0 \times x'^0_t}{p_t^0 \times x_t} = \frac{x't}{x_t}, \quad (13)$$

i.e.

$$= \frac{\text{mole fraction of } T \text{ in solution which if ideal would give observed partial pressure } p'}{\text{mole fraction of } T \text{ in solution actually giving partial pressure } p'}.$$

The actual value of the activity coefficient γ is an index of the degree of departure of the properties of the substance from the ideal, and gives some indication of the susceptibility of the substance to removal by steam distillation treatment, but it does not give a full indication. The term γ is a ratio. The pure substance T may be of very high boiling-point and therefore of low vapour pressure p_t^0 at the temperature of steam distillation; and, since $p_t = p_t^0 \times x_t$ (equation (1)), p_t will be correspondingly small when p_t^0 is small; and from equation (10)

$$p'_t = p_t \times \gamma. \quad (14)$$

Hence, even although the activity coefficient γ is relatively high, the actual partial pressure p'_t of a high-boiling substance above the solution may still be very low because the ideal partial pressure for the substance in the solution, i.e. p_t, is low.

In the study of cream deodorization the concept of the activity coefficient, γ, is useful to a consideration of the possibility of removing a known tainting substance by steam distillation; but for comparative work on the efficiency of operation of different deodorization equipment it is more convenient to think in terms of the weight concentration of taint in the steam; and in terms of the extent of removal of tainting substance effected by unit weight of steam applied to unit quantity of cream, i.e. (in factory practice) weight in pounds of steam per pound or per gallon (10 lb.) of cream (equation (9)).

Reference substances

The chemical composition of many of the substances that cause off-flavours in cream is not known. It is clear, however, that the concentration of these substances in the cream is very small. Benzyl mercaptan, for example, the causative substance of land cress taint (8), gives a distinct off-flavour when present in cream in the proportion of 1 part per 1000 million, i.e. at a concentration beyond the limits of accuracy of normal chemical estimation. Study of the operation of steam distillation units is facilitated by use of reference substances which can be added in concentrations within the range of chemical analysis, and which are easily subject to chemical estimation. Also, although much of the investigational work can be done with use of water instead of cream, it is an advantage to employ a reference substance which can be added to cream without causing objectionable flavours in the cream and butter. In order that the butter produced should be saleable the reference substances added should not be foreign to normal commercial cream and butter, i.e. they should not be adulterants. The substances diacetyl and acetoin, which are present in all creams that have served as a medium for the growth of lactic bacteria, are found in most commercial butters. They therefore may safely be added to cream as reference substances for deodorization investigations (see also (9)).

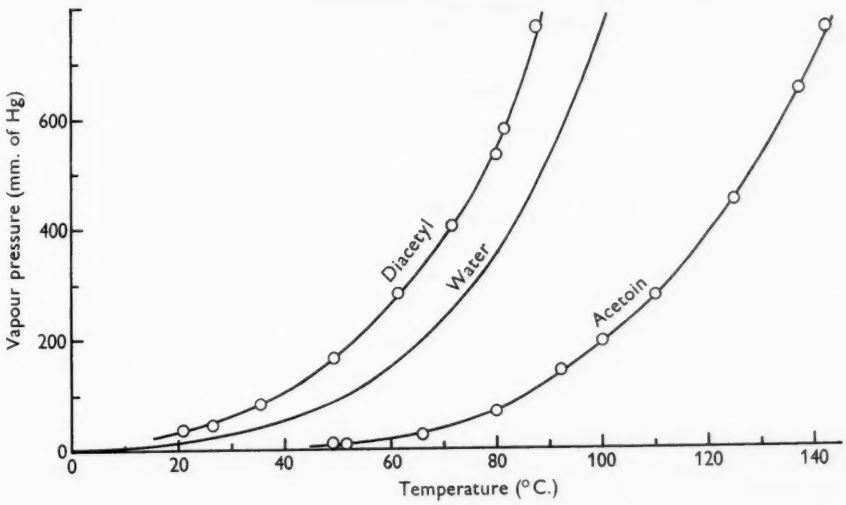

Fig. 2. Vapour-pressure/temperature curves for diacetyl and acetoin.

Properties of the reference substances

The boiling-point of diacetyl at 760 mm. pressure is 89·5° C. and of acetoin is 144° C. Since in some types of commercial cream-deodorization equipment the distillation of taint into the steam occurs either under partial vacuum or at pressures somewhat above atmospheric, some knowledge of the properties of the reference substances over a range of pressures is necessary.

(*a*) *Vapour-pressure/temperature curves.* These were determined by vacuum distillation of purified samples of diacetyl and acetoin at different reduced pressures (Fig. 2). The

vapour-pressure/temperature curve for acetoin is in close agreement with that obtained by Efron & Blom(10) by means of the isoteniscope.

(*b*) *Vapour-pressure ratios.* Equation (3) above shows that for an ideal mixture the amount of solute distilled per unit weight of steam is proportional to the value $p_t^0/p^0_{H_2O}$, i.e. the ratio of vapour pressure of the tainting substance to the vapour pressure of water. This ratio varies with change in temperature (compare Fig. 1). In Fig. 3 the vapour-pressure ratios for diacetyl and acetoin are shown both as such and as percentages of the ratio at 100° C. In conformity with the Sidgwick generalization (see above), the vapour-pressure ratio for diacetyl, which has a lower boiling-point than water, decreases, and that for acetoin, which has a higher boiling-point than water, increases, with rise in temperature. Under conditions permitting attainment of equilibrium and if Raoult's law

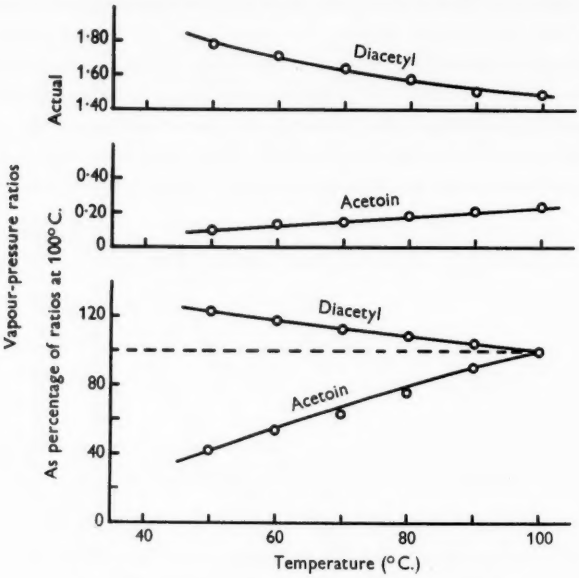

Fig. 3. Vapour-pressure ratios for diacetyl and acetoin.

were applicable, therefore, it could be expected that (*a*) the rate of removal of diacetyl from water per pound of steam used would be increased by lowering the temperature of steam distillation, i.e. by increasing the vacuum applied, and (*b*) the rate for acetoin would be increased.

Vapour/liquid equilibrium relationships for diacetyl and acetoin

For a study of the rate of removal of a substance from solution by steam distillation the term K_4 in equation (9), viz.

$$C_V^t = K_4 \times C_L^t$$

(C_V^t: concentration of taint in the vapour; C_L^t: concentration of taint in the liquid)

is an important characteristic, which, because of the wide variation of substances in dilute aqueous solution from the ideal or Raoult's law relationship, must in every instance be determined experimentally. This characteristic, termed by Virtanen & Pulkki(4) the

'distillation constant', is referred to here and in subsequent papers of this series as the 'equilibrium coefficient'. (The term 'distillation *constant*' is a misnomer, because the value may vary with variation in concentration of the substance in solution, e.g. see below, for diacetyl.)

Data for the vapour/liquid equilibrium relationship for diacetyl were not found in the literature. Data for acetoin have been published by Langlykke & Peterson (11) and by Blom & Efron (12). Langlykke & Peterson, using an iodimetric method for estimation of acetoin, determined the partial distillation values for solutions ranging from 0·005 to 0·10 %, distilled in three fractions. From their average results they computed the 'distillation constant', according to the formula of Virtanen & Pulkki (4), for the first, second and third fractions as 1·330, 1·297 and 1·298, respectively. They concluded that 'the distribution of acetoin during distillation is constant and independent of the concentration'. Blom & Efron, using an Othmer equilibrium still, obtained samples covering the whole range of composition of acetoin/water mixtures, including a number of samples with liquid composition over the range 0·045–0·960 %, and analysed them by a bichromate oxidation method. They concluded that the vapour/liquid concentration relationship was linear up to a concentration of 1·0 % in the liquid, according to the equation $C_V = 1{\cdot}345 C_L$, where C_V and C_L represent the concentrations of acetoin in the liquid and vapour, respectively. The value of the equilibrium coefficient for their individual results varied from 1·27 to 2·386. Because of this wide spread of the individual results for samples of acetoin solutions with the Othmer still some further investigation appeared to be warranted.

EXPERIMENTAL

The vapour/liquid equilibrium samples were obtained by use of the continuous equilibrium vapourization method described by McDowall (13).

The diacetyl was obtained from a commercial sample by fractional distillation at atmospheric pressure, b.p. 89–89·5°/760 mm. (uncorr.), n_D^{18} 1·3968, d_{15}^{15} 0·9920. The boiling-point differs from that quoted by Heilbron (14), viz. 88°, but is in agreement with that quoted by Schmallfuss & Rethorn (15). The refractive index and density also are somewhat higher than the values 1·3933 and 0·9904, respectively, quoted by Heilbron. Schmallfuss & Rethorn do not report on the refractive index and density of the diacetyl for which they recorded the boiling-points 89·2° (uncorr.) and 89·90 (corr.).

In the solutions diacetyl was estimated by the method of Pien, Baisse & Martin (16) as modified by Cox & Wiley (17). Before analysis all samples were diluted to a concentration within the range 0·5–5 p.p.m.

Acetoin was obtained from a commercial sample by separation of the solid dimer and washing with ether. The dimer was reconverted to acetoin as required, by warming with water. The samples of liquid and vapour were almost free from diacetyl at time of analysis. The acetoin in the sample was estimated by the Voges-Proskauer reaction according to the procedure described by Eggleton, Elsden & Gough (18). This was preferred to the iodimetric method of Langlykke & Peterson (11), because it was more readily applicable for very low concentrations of acetoin. At concentrations of acetoin above 100–150 p.p.m. the two methods gave approximately equivalent results.

RESULTS

Diacetyl. For very low concentrations of diacetyl in water the relationship $C_{\text{vapour}}/C_{\text{liquid}}$ is linear (Fig. 4A). The slope of the average line is represented by the equation $C_V = 39{\cdot}3\ C_L$. The equilibrium coefficient for pairs of samples varied from 34·0 to 43·0, but the majority of the results were within the range 37–41 (some portion of the variation must be attributed to errors of analysis). The linear relationship applies only for concentrations up to about 50 p.p.m. (see Fig. 4B). For higher concentrations the volatility of diacetyl relative to that of water decreases, as shown by the falling away of the curves in Fig. 4B, from the broken line which represents the equation $y = 39{\cdot}3x$.

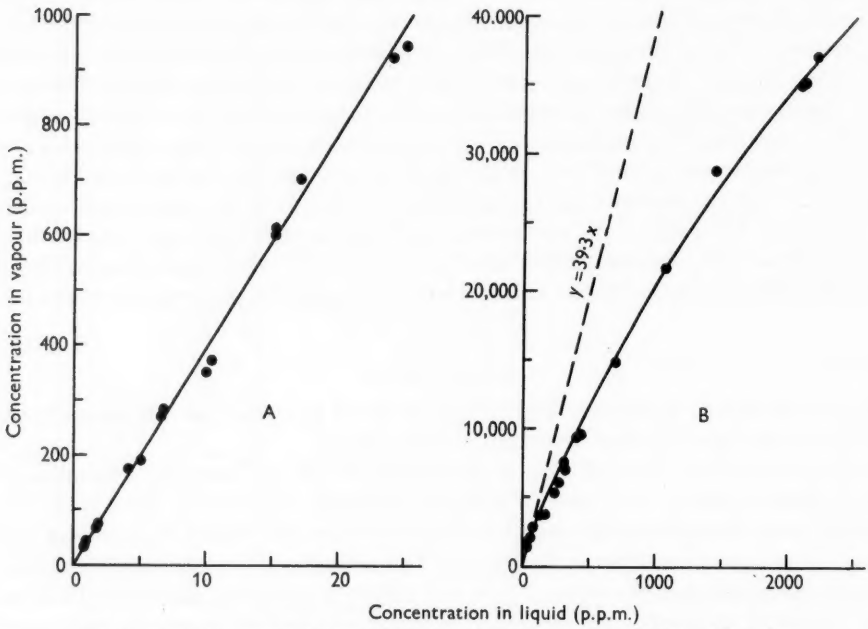

Fig. 4. Liquid/vapour equilibrium relationships for dilute solutions of diacetyl in water.

From the curves in Fig. 4 values for the fraction C_V/C_L can be read off as shown in Table 1.

Table 1. *Vapour/liquid equilibrium coefficients for diacetyl at different concentrations in water*

Concentration in liquid	20	100	200	400	800	1000	2000
C_V/C_L	39·3	32	27	23·5	21·5	20	17·2

Diacetyl is thus more suited for use as a reference substance in steam-distillation investigations in solutions which at equilibrium will give a concentration in the liquid of not more than 50 p.p.m.

Acetoin. The results are given in Figs. 5 and 6. For all concentrations up to 1·2% in the liquid the points fall close to the straight line represented by the equation $C_V = 1{\cdot}29\,C_L$. Computation of the vapour/liquid equilibrium relationships from analysis of samples

obtained by constant volume distillations of aqueous solutions of acetoin of various concentrations between 5 and 40 p.p.m. (see the results in Fig. 7) gave experimental points reasonably close to the straight line representing the equation $C_V = 1{\cdot}29\, C_L$.

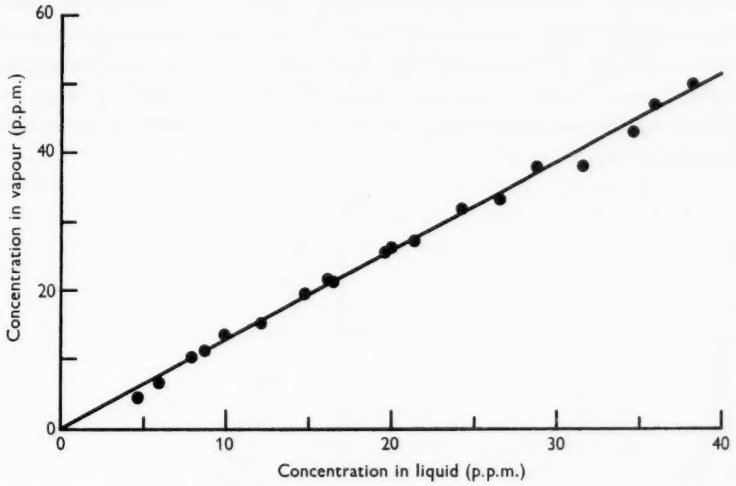

Fig. 5. Liquid/vapour equilibrium relationships for very dilute solutions of acetoin in water.

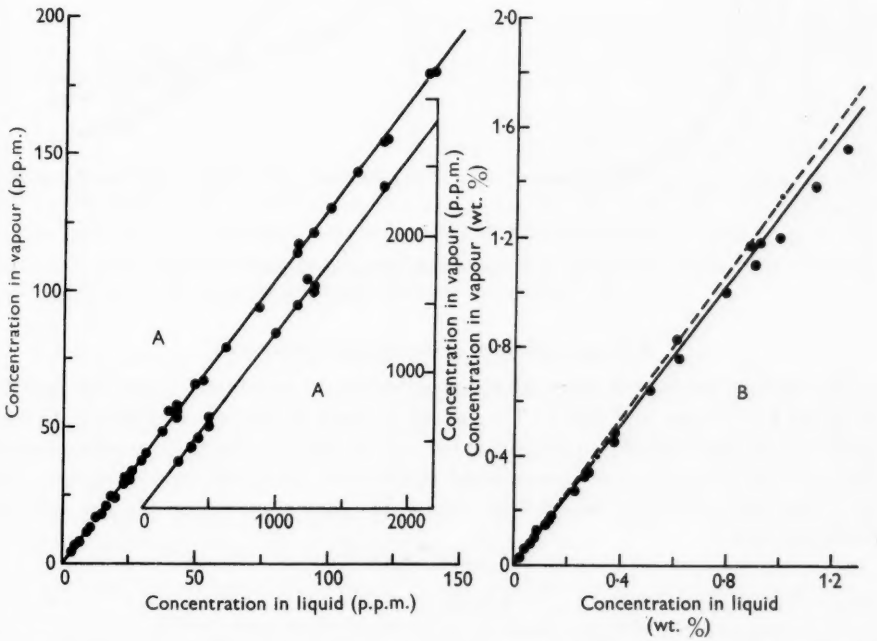

Fig. 6 Liquid/vapour equilibrium relationships for acetoin/water solutions up to 1·2% concentration. •••• Author's observed points. —— Author's equation. – – – Blom & Efron equation.

The value 1·29 for the equilibrium coefficient is in close agreement with two of the three results reported by Langlykke & Peterson (11), but is lower than the average, 1·345, found by Blom & Efron ((12); and see broken line in Fig. 6 B). As stated above, however, the results for low concentrations of acetoin from which Blom & Efron deduced their average relationships were far from consistent, and they were not very extensive. Since the continuous equilibrium vaporization method has been shown to be reliable (13), the equilibrium coefficient, 1·29, deduced from the results obtained in the present investigation, is submitted as representing more accurately than the Efron & Blom value the vapour/liquid equilibrium relationship for acetoin over the lower concentration range where Henry's law applies.

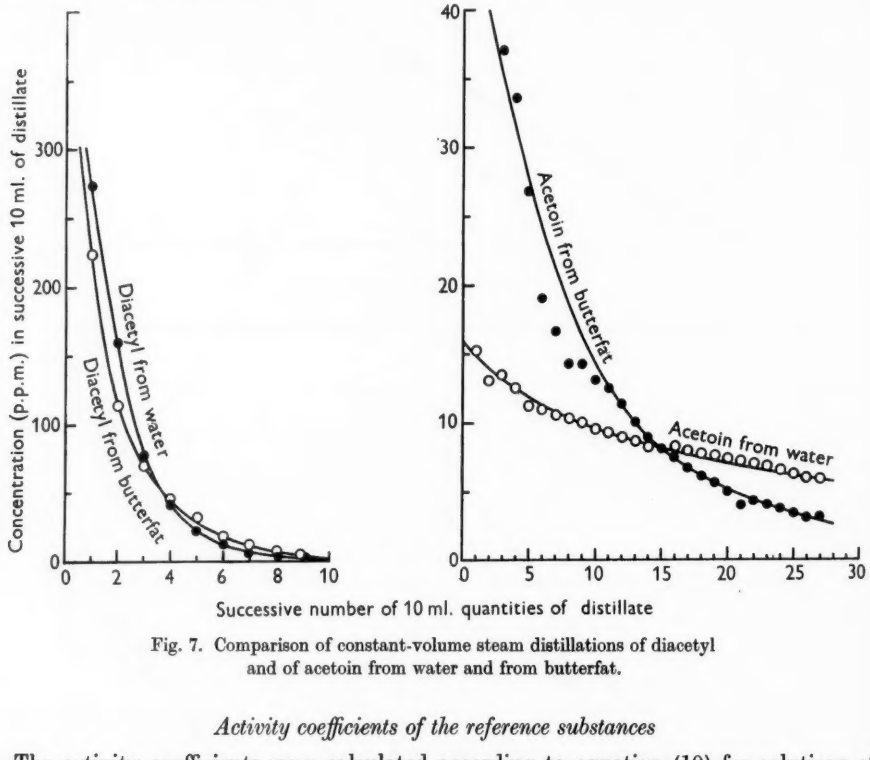

Fig. 7. Comparison of constant-volume steam distillations of diacetyl and of acetoin from water and from butterfat.

Activity coefficients of the reference substances

The activity coefficients were calculated according to equation (10) for solutions of 10 p.p.m. at 760 mm. and 100° C. The vapour pressure of pure diacetyl at 100° C. was taken as 1148 mm. and of pure acetoin as 180 mm (see Fig. 2). For the two substances at this concentration the two vapour/liquid equilibrium equations deduced above, viz. $y = 39{\cdot}3\,x$, and $y = 1{\cdot}29x$, respectively, apply. The computed values for the activity coefficient, γ, are:

diacetyl	26·4
acetoin	5·4

Diacetyl in dilute solution thus shows a much greater divergence from the ideal than does acetoin. On the other hand, the value of γ for diacetyl begins to diminish at concentra-

tions above 50 p.p.m. (compare Fig. 4B, and Table 1), whereas the value of γ for acetoin remains constant over a considerable range of concentration.

Relative solubilities of reference substances in butterfat and water

As stated above, some knowledge of the partition coefficients of the reference substances for a butterfat/water system is necessary for a study of the rate at which they are steam-distilled from cream. Although for ideal conditions the partition coefficient, i.e $C_{\text{butterfat}}/C_{\text{water}}$, would remain constant over all concentrations of reference substance, there are frequently considerable variations from the ideal, especially in very dilute solutions. For this reason measurements were made at a concentration of the same order as that used in the experimental deodorization investigations, i.e. with an overall content of 10 p.p.m. by weight in a mixture of equal weights of butterfat and water. The results, given in Table 2, show that diacetyl and acetoin are soluble in both butterfat and water.

Table 2. *Distribution of diacetyl and acetoin at overall concentrations of* 10 *p.p.m. by weight, between water and butterfat at different temperatures*

Temperature		Partition coefficients			
		Butterfat/water		Water/butterfat	
° C.	° F.	Diacetyl	Acetoin	Diacetyl	Acetoin
37·7	100	0·47	0·18	2·1	5·6
48·9	120	0·54	0·18	1·8	5·7
65·6	150	0·69	0·19	1·4	5·3
82·2	180	0·74	0·19	1·35	5·3

At 180° F., i.e. at somewhere near the normal temperature for cream deodorization, diacetyl distributes itself between butterfat and water in the ratio of 0·74:1, and acetoin in the ratio 0·19:1. Diacetyl is thus relatively more butterfat-soluble than is acetoin. With rise in temperature from 100 to 180° F. the butterfat/water coefficient for diacetyl increases from 0·47 to 0·74, whereas that for acetoin remains comparatively constant. Since most tainting substances in cream can be expected to be relatively considerably more soluble in butterfat than the two reference substances, it does not follow that results obtained in commercial practice in the deodorization of tainted cream will be in complete accord with the conclusions reached from a study of the rate of removal of the selected reference substances from water or from cream.

Comparison of distillation of reference substances from water and butterfat

It has been stated earlier that thermodynamically* it can be shown that the vapour pressure of a substance over butterfat is the same as that over water when the butterfat and water are in contact and the substance is in partition equilibrium between them. This is illustrated by the curves given in Fig. 7, which represent the diacetyl and acetoin contents of each 10 ml. of distillate taken from 500 ml. of water and from 500 ml. of butterfat containing 10 p.p.m. of diacetyl or acetoin.

* E.g. the conventional illustration, as follows: Consider butterfat and water containing diacetyl are in contact, in partition equilibrium, one to each side of a U-tube and that the limbs of the U-tube are joined by a connecting tube. If the vapour pressures of diacetyl above the water and butterfat were not equal there would be a continuous circulation of diacetyl from the region of higher vapour pressure to the region of lower vapour pressure, with continuous re-establishment of the partition equilibrium across the interface—a result which would contravene the law of conservation of energy.

The amount of heat required in a heating mantle just to keep water refluxing to the outlet of a lagged flask, or to keep butterfat at 102° C., was determined by a prior run, the control being made by means of a Variac transformer. The flask was lagged and, with the mantle heating at this rate, steam, dried by means of a cyclonic separator bulb just before entry, was blown in at a constant rate, and the condensate was collected in 10 ml. quantities for analysis.

It will be seen from Fig. 7 that the rate of removal of diacetyl from butterfat was approximately the same as the rate of removal from water, in reasonable agreement with the finding (see Table 2) that at 100° C. the partition coefficient for diacetyl/water would be 0·87. The rate of removal of acetoin from butterfat, on the other hand, was several times greater than the rate from water. This may be related to the finding (Table 2) that the partition coefficient for acetoin/water at 100° C. would be approximately 0·20, and therefore the vapour pressure of acetoin above a solution of acetoin in butterfat would be much higher than above a solution of the same concentration in water. The rate of distillation from butterfat was not quite so high as would be expected from the partition coefficients, but the steam bubbles passing through the butterfat probably did not become saturated with reference substance.

SUMMARY

The rate of removal of a dissolved tainting substance from cream by steam-distillation treatment is dependent in part on the relative volatility (or boiling point) of the pure substance, in part on the temperature of steam distillation, and in part on the influence of water on the vapour pressure of the tainting substance in dilute solution (i.e. on the activity coefficient, γ).

The application of Raoult's law and Henry's law to the problem of removal of taints from cream is discussed. For the dilute solutions commonly encountered in cream Henry's law can be expected to apply.

The equilibrium relationships for diacetyl and acetoin used as reference substances in investigations on cream-deodorizing equipment have been determined. The equilibrium coefficient for diacetyl in water at concentrations up to 50 p.p.m. in the liquid, as measured by the continuous equilibrium vaporization method, is 39·3. With rise in concentration above 50 p.p.m. in the liquid the coefficient decreases. At 100 p.p.m. it is 32 and at 1000 p.p.m. it is 20. The coefficient for acetoin in water at all concentrations up to 1·2% in the liquid is constant at 1·29. The activity coefficient for diacetyl in concentrations up to 50 p.p.m. was found to be 26·4 and for acetoin at concentrations up to 1·2%, 5·4.

For diacetyl the solubility partition coefficient as between butterfat and water rises from 0·47 at 100° F. to 0·74 at 180° F. For acetoin the partition coefficient remains constant at 0·18–0·19 over this range of temperatures. Correspondingly diacetyl is removed equally easily from butterfat and from water, whereas acetoin steam-distils much more readily from butterfat than from water.

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597. MICROSCOPIC APPEARANCE OF FAT ON THE MILK SURFACE AS AFFECTED BY MECHANICAL DISTURBANCE OF THE SURFACE

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(With 1 Figure)

An insight into the inner nature of the surface phenomena associated with dairy processes may be acquired quantitatively by measuring the surface or interfacial tension and the surface viscosity (cf. Mohr & Brockmann (11,12,13)). The figures so obtained give an expression for the interplay of surface forces caused by different capillary active components of milk—proteins, phospholipids, triglycerides, free fatty acids, etc. On the other hand, a qualitative picture of the milk surface obtained by examining the free surface of milk under the microscope in incident light may also be of advantage, particularly when dealing with phenomena involving the milk fat, which usually appears in microscopically perceptible forms (agitation, churning, whipping, etc.). In fact, this qualitative method has been of use in studying the churning process, homogenization, etc. (King (4,5,8)). Dependent upon the conditions (temperature of milk, extent of agitation the milk has received), the fat on the milk surface may appear as more or less numerous black globules (below 15–20° C.) or as bright lenses (above 25–30° C.). The globules may be cemented together to clumps by means of a zone of the liquid-fat fraction spread out from the globules, while another part of this spread-out fat may appear as thin patches on the surface. Very probably the milk surface between the globules, clumps and patches is covered by a microscopically invisible fat film, only one or a few molecules thick (4). On the application of two-dimensional compression it is possible to concentrate this very thin film on the surface of skim milk to microscopically visible fat specks (6), while in whole milk the two-dimensional compression provokes also clumping of the fat globules.

EXPERIMENTAL METHOD

In the microscopic investigations of the milk surface referred to above, a large drop of milk was taken by means of a glass rod or a pipette, placed on a slide, preferably provided with a cavity, and the uncovered surface examined in incident light. However, a drop thus taken would give a picture of a disturbed milk surface.

In order to obtain a milk surface as fresh and genuine as possible, in the present investigation an overflow outfit was used. In this apparatus (Fig. 1) which represents a modification of the Röntgen double funnel (14), the milk or cream rises in the bowl of an inner funnel, and after filling it flows over its rim into an outer concentric funnel from which it is drawn off. The device is made as low as possible to fit it on the microscope stage under the objective. It is clamped by means of a simple holder into the mechanical stage, and hence the surface of milk in the inner funnel can be conveniently moved in the view field of the microscope.

For the microscopic examination in incident light a simple, inexpensive prism vertical illuminator (C. Baker, London), in conjunction with a short mounted objective (to bring the back lens as near as possible to the vertical illuminator), was used. A magnification of approximately 150 times was achieved with the objective 10× (numerical aperture 0·28), binocular tube 1·5× and the eyepieces 10×. A Cooke, Troughton & Simms high-power microscope lamp served as the light source. This optical outfit, although simple, gave a very clear image of the milk surface. The fat globules and clumps occurring on the surface were counted by means of a calibrated net eyepiece micrometer, usually in 10 view fields. The diameters relating to the fat globules on the surface are not the real diameters of the globules, but they express the diameters of the spherical segments ranging above the surface. They thus vary for a particular globule depending upon its degree of immersion. On the other hand, the globules may be more or less stretched to lenses.

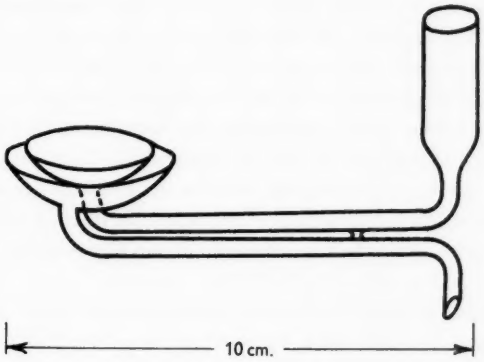

Fig. 1. Overflow outfit for the microscopic investigation of freshly formed milk surface.

RESULTS

The surface of large milk drops taken by means of a glass rod or a pipette contains a number of fat globules, clumps or lenses. According to earlier findings of the author (4) the surface of freshly drawn milk (about 15 min. after milking) at a temperature of 31–34° C. contained less than 500 fat lenses per sq.mm. The same milk held overnight at 12–14° C. showed approximately 500 fat globules per sq.mm. of surface. About the same figure was observed in the case of a large number of milk samples at a temperature of 10–14° C. With an increase of the milk temperature to about 20° C. the number of fat globules and clumps on the surface increased to about 2000–2500 per sq.mm. However, in the immediate vicinity of the surface the milk fat globules were densely crowded.

When a few c.c. (4–6) of milk or cream are allowed to overflow the inner funnel of the outfit described above, the milk surface in the inner funnel appears almost free from microscopically visible fat (globules, clumps, patches, lenses). On scanning the surface by means of the mechanical stage only in some occasional view fields one or two globules may be encountered. The surface may remain more or less free of fat during about 1 hr. Below the surface, very close to it, the fat globules are, however, crowded in large numbers as

revealed in the half-shadow zone around the bright view field when drawing closer the iris diaphragm of the light source. The picture is nearly the same in a temperature interval between 10 and 40° C.

If the fresh milk surface containing no visible fat is disturbed, a number of fat globules, clumps, lenses or patches appear there. One way of doing this is to touch the surface with a platinum wire or ring. At temperatures below 20° C., the fat appears on the milk surface as black globules, which at the lower temperatures—about 10° C.—exhibit a slight depression around them suggesting the existence of a rather large contact angle between the fat and plasma. The diameter of the spherical segments of globules ranging above the surface is about 3–8 μ.

When the temperature rises above 20° C. the globules become surrounded by a narrow bright zone which consists of the liquid fat fraction spread out from the globule. Here also the diameter of the globules on the surface is about 3–8 μ. It is of interest that smaller fat globules with a diameter below 2–3 μ are almost completely absent from the surface. This presumably explains the fact that during churning the smaller globules are left behind in the butter-milk, and predominantly larger ones are transferred into the butter grain. A number of globules are joined together to clumps by means of the zone of liquid fat surrounding them, while a portion of the liquid fat detached from the globules appears as thin specks and patches on the milk surface. The black fat globules which come to the surface after the first few touches tend to drift slowly apart from each other. Possibly this is caused by the spreading out of the liquid fat fraction from the globules onto the surface, as a very thin, invisible film, which on expanding takes the fat globules with it (cf. King (7)).

At temperatures in the vicinity of 30° C. more and more of the fat in the globules melts, and the black globules are stretched by surface forces to bright lenses which show interference colours on their periphery. The milk surface now contains predominantly fat lenses and patches apart from some globules and clumps still left. The diameter of lenses and globules ranges between 7 and 15 μ.

At a low degree of disturbance the influence of temperature on the amount of fat on the surface was not conspicuous, e.g. five touches with a platinum loop at 10° C. brought about 50 globules to a view field, at 20° C. about 60 globules and at 30° C. about 60 globules, clumps and lenses. The surface covered with fat may be easily cleaned and rendered free of visible fat by overflowing a few c.c. of milk or cream.

In the experiments described below a platinum ring 3·5 mm. in diameter was immersed into the milk and lifted again, as far as possible on the same spot of the surface, inside the view field of the objective.

Table 1 demonstrates the effect of the extent of disturbance caused by touching the milk surface with a platinum ring on the number of the fat globules and clumps brought on the surface as influenced by the fat content of milk or cream. The milk and creams were unpasteurized. The creams of medium fat content were obtained from the 39% cream by diluting with skim milk. The samples were held overnight in a refrigerator, their temperature being about 2° C. The experiment was carried out at 15–16° C. In the case of milk and up to 50 touches only fat globules appeared on the surface, while in all other cases besides the globules clumps were also present.

As seen from this table the number of fat globules and clumps penetrating the milk surface rapidly increases with the extent of disturbance. There is a marked increase in the

Table 1. *The effects of the extent of disturbance (by touching the milk surface with a platinum ring) and of the fat content of milk or cream on the amount of fat brought to the surface*

	No. of fat globules and clumps per sq.mm. of the milk surface			
No. of touches	4·5% fat	9·7% fat	19·5% fat	39% fat
0	0	0	0	0
5	180 globules	230 globules and clumps	230 globules and clumps	2450 globules and clumps
10	1590 globules	1550 globules and clumps	1360 globules and clumps	5640 globules and clumps
15	2090 globules	2360 globules and clumps	3590 globules and clumps	5640 globules and clumps
20	2270 globules	3820 globules and clumps	4450 globules and clumps	7000 globules and clumps
25	2860 globules	—	—	8550 globules and clumps
50	4000 globules and clumps	—	—	—

Table 2. *The effect of ageing on the number of fat globules and clumps brought to the surface*

	Fresh milk and cream Measuring temperature 18–19° C. No. of fat globules, lenses and clumps per sq.mm. of milk surface				Milk and cream aged 17 hr. at 4° C. Measuring temperature 18–19° C. No. of fat globules and clumps per sq.mm. of milk surface				Milk and cream aged 34 hr. at 4–6° C. Measuring temperature 18–19° C. No. of fat globules and clumps per sq.mm. of milk surface			
No. of touches	4·2% fat	10·8% fat	21·5% fat	43% fat	4·2% fat	10·8% fat	21·5% fat	43% fat	4·2% fat	10·8% fat	21·5% fat	43% fat
0	0	0	0	0	0	0	0	0	0	0	0	0
5	180	230	140	360	90	270	320	1090	90	180	180	550
10	360	450	1270	1640	90	1000	1450	2180	410	1680	410	3550
15	770	950	2230	2550	820	2360	3680	3640	1230	3320	3230	4180
20	1140	1450	—	3550	1450	1820	3770	4590	1590	3640	4230	4910

quantity of fat brought on the surface with higher fat percentages, particularly in the case of 39% cream.

Table 2 shows the effect of ageing of milk or cream on the number of fat globules and clumps brought to the surface by disturbing it with a platinum ring. The milk as well as the creams was unpasteurized.

A definite although slight increase in the number of fat globules and clumps brought to the surface may be noticed with ageing, especially in the case of more intensely disturbed surfaces (20 touches).

Concerning the effect of the pH value of milk, Table 3 reveals a distinct minimum in the number of fat globules transferred to the milk surface at pH 5·3 in the pH range between 4·8 and 7·8. The minimum is more pronounced at a more intense disturbing of the surface (15 and 20 touches). The milk used was unpasteurized and held overnight in a refrigerator. The pH was adjusted by adding 0·1 N-sodium hydroxide or diluted lactic acid to the milk.

Table 3. *The effect of* pH *on the amount of fat brought to the milk surface by touching it with a platinum ring. Measuring temperature* 16–17° C.

No. of touches	No. of fat globules per sq.mm. of milk surface			
	pH 7·8	pH 6·7	pH 5·3	pH 4·8
0	0	0	0	<50
5	180	180	90	450
10	910	820	730	950
15	2270	1410	1090	1450
20	2000	1640	1180	1640

As appears from Table 4, the character of the surface of the platinum ring, whether hydrophilic (flamed) or hydrophobic (paraffin-coated), does not affect the transfer of the fat globules into the milk surface. The milk used was pasteurized.

Table 4. *The effect of the surface character of the ring on the amount of fat on the milk surface. Measuring temperature* 21–22° C.

No. of touches	No. of fat globules and clumps per sq.mm. of milk surface	
	Flamed platinum ring	Paraffin-coated platinum ring
0	<50	0
5	180	500
10	730	500
15	1590	1500

Another way of disturbing the milk surface and bringing the fat globules to it is to allow air bubbles to burst in the milk in the overflow funnel. When, for example, the cream put through the overflow outfit contains larger amounts of air—which occurs at higher fat contents—a number of the air bubbles reaching the surface explode there, throwing the fat globules, clumps and patches attached to their inner surface on to the cream surface. Other bubbles, more resistant, remain underneath the surface, their tops appearing as iridescent circles, or as grey circles surrounded with an iridescent fringe on the cream surface.

The bursting of the bubbles may also be achieved by blowing air through the milk in the

overflow funnel by means of a fine capillary jet immersed under the milk surface layer. Generally, the first bubbles collapse very quickly; with time the bubbles, however, become more stable. On the bursting of the first few bubbles the fat thrown on the milk surface appears there as a circular area.

In this case also the appearance of the fat on the surface depends upon the temperature, while the amount of this fat is associated with the extent of disturbance, viz. the number of bubbles burst. For example, the bursting of some few bubbles (2–3) in the milk at 10–15° C. brought about 300 black globules on 1 sq.mm. of the surface, the diameter of the globules ranging between 3 and 8μ. Bursting of more bubbles (about 10–15) caused appreciable increase in the number of the globules and small clumps, about 7000 globules and clumps appearing per sq.mm. At 20° C. the number of globules and clumps after bursting of some few bubbles (2–3) was approximately 200 per sq.mm., after bursting of more bubbles (about 10–15) about 5000 per sq.mm. At 30° C. the bursting of some few bubbles brought about 400 globules and lenses per sq.mm. of the surface, while after more bursts (about 10–15) the surface became densely crowded with fat. Besides larger fat patches, there occurred very small ones, representing the source of finely dispersed fat in agitated milk. The fact that most of the fat at this temperature is liquid can be easily demonstrated by sprinkling traces of fat dyes (Sudan III or IV, Sudan Black) on the surface. The lenses and patches take up the stain. When a loopful of milk is taken from the surface and examined in transmitted light, an excessive clumping may be observed. Staining with the above-mentioned fat stains reveals that the fat forms an almost continuous blanket on the milk surface. The coloration starting from a dye particle gradually spreads over the whole blanket.

DISCUSSION

The relative absence of fat on the undisturbed surface of milk and cream in the overflow apparatus reported above, indicates that the fat emulsion is fairly well stabilized, even at as high fat content as 43%. On the other hand, the easy accumulation of visible fat on the surface of milk or cream by disturbing the surface layer indicates that under certain circumstances the protection afforded by the fat-globule membrane may be appreciably weakened. The inner mechanism of the changes in the fat-globule surface due to the above-mentioned actions still remains to be disclosed.

It appears that once the fat globules are wholly submerged in the milk plasma they are not able to rise spontaneously to a quiescent surface; but if brought on to the surface they float there and do not spontaneously submerge. This is in accord with the observations of van Kreveld (9) that fat clumps are not able to penetrate the milk surface. However, when caught by the surface during its formation the clumps stick to the surface.

The observed agglomeration and dispersion of the fat on the milk surface is probably closely associated with the lipolysis which takes place in raw milk which has been agitated in the presence of air. According to Krukovsky & Sharp (10), agitating cold raw milk (2° C.) did not induce lipolysis, while pronounced lipolysis—as estimated by the increase in titratable acidity and by decrease in pH—occurred at 25° C. and above. Kelley & Dunkley (3) observed minimum activation by agitation at 20° C. This activating effect of agitation on lipolysis has been attributed to an alteration of the surface characteristics of the fat

globules connected with the disruption of the globule membrane (Krukovsky & Sharp(10), Dunkley & Smith(1)). As shown in the present investigation the fat globules affected by the disturbing of the surface layer of milk become hydrophobic, presumably due to the damaging of their membrane, which makes possible a partial or total coverage of the globule surface by the liquid fat. This unprotected fat will be readily accessible to the action of lipolytic enzymes occurring in the milk plasma. Besides, the liquid fat in thin patches occurring either on the free surface of agitated milk or on the surface of air bubbles at temperatures above 20–25° C., also would be exposed to the lipase. The more violently the surface layer of milk is disturbed, the more unprotected fat is transferred on to the surface and made available to the action of lipase. On the other side, increase of temperature renders more of the fat liquid. The role of temperature is clearly demonstrated by the observation reported above that on bursting of about 10–15 air bubbles in the overflow outfit at 20° C., the fat globules and clumps on the milk surface occur separately, while at 30° C. the milk surface is covered by an almost continuous blanket of unprotected liquid fat. This is in accord with the statement of Herrington(2) that the sensitivity of milk to activation by agitation increases rapidly as the temperature is increased up to 24–29° C. The activation of lipolysis by agitation has recently attracted considerable attention in connexion with rancidity caused by pipeline milkers, especially by 'risers' in the pipe lines (Herrington(2), Kelley & Dunkley(3)).

SUMMARY

The freshly formed surface of milk or cream obtained by overflowing in a double funnel is nearly free from microscopically visible fat.

When this surface is disturbed, either by repeated touching with a platinum ring or by allowing air bubbles to burst there, the fat globules and clumps are brought on to the surface, appearing there as such, or being stretched to lenses, or being spread as patches.

The amount of fat brought onto the surface increases with the extent of disturbance, viz. the number of touches with a platinum ring or the number of air bubbles burst, and with the fat percentage of cream.

Ageing of milk or cream slightly promotes the transfer of fat globules onto the surface.

At 30° C. the bursting of about 10–15 air bubbles is enough to cover the milk surface in the overflow outfit with an almost continuous fat blanket, while at 20° C. the globules and clumps occur separately.

The fat globules rendered hydrophobic by mechanical treatment of the milk surface, as well as the fat patches on the surface, are likely to be accessible to the action of lipase. This may explain the agitation activation of lipolysis in raw milk during certain stages in machine milking and during transportation.

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598. A COMPARISON OF THE GERBER AND RÖSE GOTTLIEB METHODS FOR THE DETERMINATION OF FAT IN MILK

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(With 1 Figure)

It has been known for some years that the Gerber test for the fat content of milk tends to give higher results than the Röse Gottlieb gravimetric method, but the many papers that have dealt with this subject since 1929 (1) have given widely varying estimates of the magnitude of the discrepancy. The accuracy of the Gerber test has received increased attention of recent years, and Hostettler & Hänni (2), Roeder (3) and Radema & Mulder (4), among others, have given additional data and reviewed the literature.

Radema & Mulder (4), from the results of the examination of about 170 samples of milk by the two methods, found the following relationship between the Gerber percentage (G) and the Röse Gottlieb percentage (RG): $G = 1{\cdot}040\ RG - 0{\cdot}070$. On this basis they recommended (5) that the capacity of the Gerber milk pipette be reduced from the specified 11·00 ml. to 10·77 ml., as this would ensure agreement between Gerber and Röse Gottlieb results for milk of 3·75% fat content, the average for milk in the Netherlands, and only a very small, and virtually negligible, discrepancy over a wide range of fat content. This recommendation was incorporated in a new Netherlands Standard Specification, N 962 (6), for the Gerber test.

The Scientific Committee responsible for this specification drew attention to the fact that improving the accuracy of the Gerber test in this way leaves the calibration of the butyrometer unchanged, so that it may be considered as an international instrument, whilst 'the capacity of the milk pipette may be adapted to national or regional needs provided its volume... be practically chosen in accordance with the international gravimetric basic method (Röse Gottlieb)'.

Following this lead, other countries—Eire, France, Germany and New Zealand—have the matter under consideration at present.

Members of the Committee of the British Standards Institution responsible for revision of the specification dealing with the Gerber method (B.S. 696:1936) have also conducted an investigation, and their findings are briefly reported in this paper.

EXPERIMENTAL PROCEDURE

Preliminary work in which samples from the same bulk milks were circulated to a number of laboratories represented on the Committee was unsatisfactory, as owing to churning of the fat the samples did not arrive in a suitable condition for accurate testing. For this reason, in the subsequent investigation samples of milk were obtained locally by the

participating laboratories. A typical amyl alcohol, which satisfied the requirements of B.S. 696:1936, was supplied to the laboratories. Each laboratory undertook to examine 15–20 samples of milk, covering the range of about 2·5–6·0% fat, during each of the periods October/November 1952, February/March 1953, and May/June 1953. These periods were selected to represent different seasons of the year and the related variations in the feeding of the dairy herds.

The Gerber tests were carried out in accordance with B.S. 696:1936, using checked, accurate butyrometers and pipettes. Each sample was tested in quadruplicate, and the butyrometer readings were taken to 0·02%. The Röse Gottlieb determinations were made, in duplicate, by the technique described in B.S. 1741:1951. The Chemistry Department of the National Institute for Research in Dairying, and the Central Laboratories of United Dairies Ltd., the Nestlé Company Ltd., and the Milk Marketing Board co-operated in this investigation for the three periods.

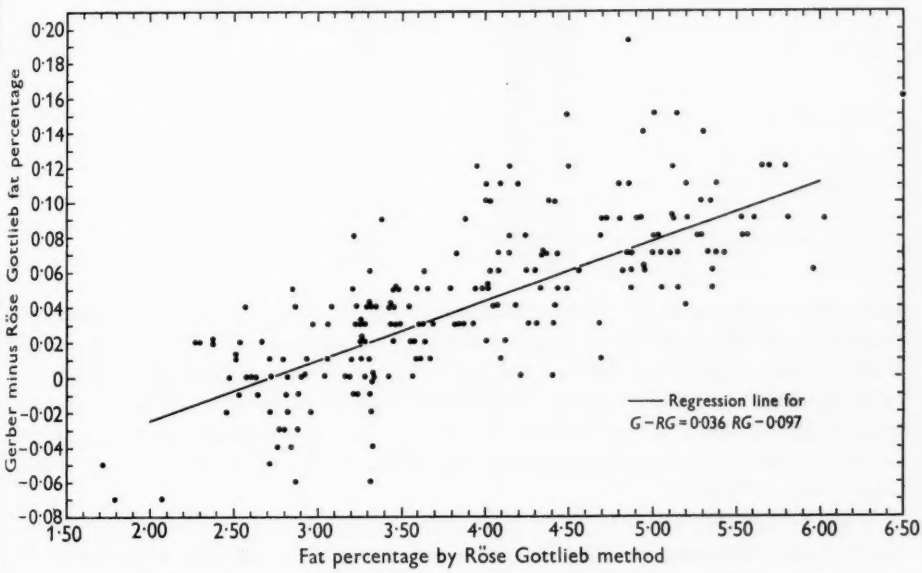

Fig. 1. Difference between Gerber and Röse Gottlieb fat percentages for 208 milk samples.

RESULTS

The results for the total of 208 milk samples for the three periods have been examined statistically and the relationship between the Gerber and Röse Gottlieb fat percentages is given by the following regression equation:

$$G = 1{\cdot}036\,RG - 0{\cdot}097,$$

where G = the Gerber percentage and RG = the Röse Gottlieb percentage. The correlation coefficient is +0·9995.

In Fig. 1, for each individual sample, the difference between the percentages found by the two methods ($G - RG$) has been plotted against the Röse Gottlieb percentage, and the regression line is shown.

The results obtained during each of the three periods have been examined separately; they did not show any significant seasonal differences in the relationship between Gerber and Röse Gottlieb results.

DISCUSSION

From the regression equation, the average differences between Gerber and Röse Gottlieb percentages ($G - RG$) have been calculated for the various levels of Gerber fat percentage. These values, given in Table 1, show that for normal levels of fat the accuracy of the Gerber

Table 1. *Average differences between Gerber and Röse Gottlieb fat percentages when using the Gerber milk pipette of* 11·04 *ml. capacity as specified*

Range of fat % by Gerber test	Difference, Gerber % minus Röse Gottlieb %
1·98–2·26	−0·02
2·27–2·55	−0·01
2·56–2·84	Nil
2·85–3·13	+0·01
3·14–3·41	+0·02
3·42–3·70	+0·03
3·71–3·99	+0·04
4·00–4·28	+0·05
4·29–4·56	+0·06
4·57–4·85	+0·07
4·86–5·14	+0·08
5·15–5·43	+0·09
5·44–5·72	+0·10
5·73–6·00	+0·11
6·01–6·29	+0·12

test would be improved by reducing the capacity of the milk pipette. The average fat content of the milk produced in England and Wales is approximately 3·6%, and at this level the average difference between the results by the two methods would be nil if the capacity of the Gerber milk pipette were reduced from 11·04 ml. of water (as at present specified in B.S. 696:1936) to 10·94 ml. If this were done, the average difference between Gerber and Röse Gottlieb percentages for the various levels of Gerber percentage would be as given in Table 2.

It will be seen that with a 10·94 ml. pipette the average error of a Gerber fat percentage will, for most purposes, be negligible over a wide range of fat content. For example, it

Table 2. *Average differences between Gerber and Röse Gottlieb fat percentages when using a Gerber milk pipette of* 10·94 *ml. capacity*

Range of fat % by Gerber test	Difference, Gerber % minus Röse Gottlieb %
1·87–2·25	−0·04
2·26–2·63	−0·03
2·64–3·02	−0·02
3·03–3·40	−0·01
3·41–3·79	Nil
3·80–4·17	+0·01
4·18–4·56	+0·02
4·57–4·95	+0·03
4·96–5·33	+0·04
5·34–5·72	+0·05
5·73–6·10	+0·06
6·11–6·49	+0·07

would be within 0·01 for 3·03–4·17% fat, 0·02 for 2·64–4·56% and 0·04 for 1·87–5·33%; and when it is desired for special purposes a correction may be applied to the Gerber reading in accordance with Table 2.

Comparison of our regression equation ($G = 1{\cdot}036\ RG - 0{\cdot}097$) with that obtained by Radema & Mulder (4) in Holland ($G = 1{\cdot}040\ RG - 0{\cdot}070$) using the Gerber technique of the Netherlands Specification N 910, shows that the differences which we have found between the Gerber and Röse Gottlieb percentages are smaller than their differences by about 0·04 at each level of fat.

After the investigation that has been described had been completed, the Technical Chemistry Department of the Hannah Dairy Research Institute carried out a similar comparison of the Röse Gottlieb and Gerber methods on thirty-eight samples of milk taken during November and December 1953, and found the following relationship: $G = 1{\cdot}032\ RG - 0{\cdot}080$. It is gratifying to observe that this equation, obtained on milks in Scotland, is in very close agreement with our equation in its assessment of the difference between Gerber and Röse Gottlieb results.

SUMMARY

The Gerber method (B.S. 696:1936) and the Röse Gottlieb method (B.S. 1741:1951) for the determination of the fat content of milk have been compared on 208 samples of milk covering the range of 2·5–6·0% fat. The following relationship was found between the Gerber percentage (G) and the Röse Gottlieb percentage (RG): $G = 1{\cdot}036\ RG - 0{\cdot}097$. Thus, for milks containing 3·0, 4·0, 5·0 and 6·0% fat, as determined by the gravimetric Röse Gottlieb method, the Gerber test gave results which were higher, on the average, by 0·01, 0·05, 0·08 and 0·12% fat respectively.

If the capacity of the Gerber milk pipette were reduced from 11·04 ml. of water, as specified at present, to 10·94 ml., the average results obtained by the two methods would be in agreement for milks with a fat content around 3·6%, the average for milk in England and Wales, and the difference between the results by the two methods would, for most purposes, be negligible over the normal range of fat content.

Tables are given, for the 11·04 and 10·94 ml. milk pipettes, showing the average differences between the results by the two methods for various values of Gerber fat percentage.

This work was carried out on behalf of the British Standards Institution, and the authors wish to thank the scientific committee responsible for their assistance and advice during the course of the investigation. They also wish to thank the organizations in whose laboratories the work was carried out, and their colleagues who rendered analytical assistance.

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637.127.683

599. THE LACTOSE-CHLORIDE CONTRIBUTION TO THE FREEZING-POINT DEPRESSION OF MILK

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(With 2 Figures)

Several previous attempts have been made to assign definite fractions of the total freezing-point depression of milk to the lactose and chloride present. For example, the results of Coste & Shelbourn (1) and Porcher & Chevallier (2) with respect to these constituents are summarized in the following table:

Component	Concentration	Freezing-point depression (° C.)	Reference
Alkali chlorides	0·1% as chloride ion	0·110	Coste & Shelbourn
Lactose	4·7%	0·250	
Alkali (Na + K) chlorides	0·18% (chloride ion 0·099%)	0·111	Porcher & Chevallier
Lactose	5·0%	0·293	

It will be seen that the fraction allocated to chlorides is very much the same in both cases, but there is a big difference in the values given for lactose, 0·053 and 0·059° C. respectively being allocated for each 1% of the sugar.

It is not clear whether these figures were obtained experimentally, or were calculated using the molar freezing-point depression constant. The theoretical depression caused by 5% hydrated lactose in water is 0·272° C., but Porcher & Chevallier (1923) report 0·293° C. for the same concentration in milk. However, correction for the weight of other constituents (assuming fat 4·0% and protein 3·5%) increases the theoretical depression for milk to 0·294° C. For this reason it is difficult to interpret the figure given by Coste & Shelbourn (1919). It was decided, therefore, to check these results using a direct approach to the problem. The contributions of the lactose and chlorides to the total freezing-point depression were determined by a process of incremental addition. By adding known amounts to watered milk, a direct correlation was obtained between the amount added and the freezing-point depression in a medium substantially the same as the original. Addition of either lactose or chlorides over a series of concentrations gave a straight line relationship between the increased freezing-point depression and the amount of solid added.

With the difficulty experienced in dissolving lactose, it was convenient to use milk containing 50% added water for the sugar, but much less (about 20%) was added for the chlorides.

EXPERIMENTAL

Freezing-point depressions were measured in a Hortvet cryoscope.

Lactose was determined according to the *Methods of the Association of Official Agricultural Chemists*, 7th edition, 1950, p. 232.

Chlorides were determined by the method of Davies (3).

A. *Effect of lactose*

An equal weight of water was added to each milk sample. To separate portions, lactose (monohydrate) was then added, using the quantities shown in Table 1, and the sample stirred until solution was complete. It will be noted that the amount added was increased until the freezing-point depression was slightly greater than that of the original milk. Results of four experiments are given in Table 1 and the relationship between the lactose added and increase in freezing-point depression is shown in Fig. 1.

Table 1. *Relationship between added lactose and increase in freezing-point depression*

Series	Sample	Lactose added (g.)	Total lactose found (%) (as monohydrate)	Freezing-point (° C.) (Hortvet)	Increase in lactose conc. (%)	Increase in freezing-point depression (° C.)
A	1. Original milk	—	4·74	−0·538	—	—
	2. 150 g. milk, 150 g. water	—	2·37	−0·270	—	—
	3. 150 g. milk, 150 g. water	10·5	5·74	−0·467	3·37	0·197
	4. 150 g. milk, 150 g. water	12·0	6·24	−0·494	3·87	0·224
	5. 150 g. milk, 150 g. water	13·5	6·64	−0·520	4·27	0·250
	6. 150 g. milk, 150 g. water	15·0	7·19	−0·550	4·82	0·280
B	1. Original milk	—	4·95	−0·541	—	—
	2. 150 g. milk, 150 g. water	—	2·50	−0·272	—	—
	3. 150 g. milk, 150 g. water	10·5	5·86	−0·465	3·36	0·193
	4. 150 g. milk, 150 g. water	12·0	6·35	−0·493	3·85	0·221
	5. 150 g. milk, 150 g. water	13·5	6·77	−0·524	4·27	0·252
	6. 150 g. milk, 150 g. water	15·0	7·30	−0·552	4·80	0·280
C	1. Original milk	—	4·95	−0·544	—	—
	2. 150 g. milk, 150 g. water	—	2·49	−0·271	—	—
	3. 150 g. milk, 150 g. water	10·5	5·88	−0·471	3·39	0·200
	4. 150 g. milk, 150 g. water	12·0	6·37	−0·497	3·88	0·226
	5. 150 g. milk, 150 g. water	13·5	6·78	−0·524	4·29	0·253
	6. 150 g. milk, 150 g. water	15·0	7·28	−0·552	4·79	0·281
D	1. Original milk	—	4·82	−0·537	—	—
	2. 150 g. milk, 150 g. water	—	2·42	−0·268	—	—
	3. 150 g. milk, 150 g. water	10·5	5·80	−0·463	3·38	0·195
	4. 150 g. milk, 150 g. water	12·0	6·28	−0·491	3·86	0·223
	5. 150 g. milk, 150 g. water	13·5	6·70	−0·519	4·28	0·251
	6. 150 g. milk, 150 g. water	15·0	7·23	−0·550	4·81	0·282

B. *Effect of chlorides*

Similarly, the effect of chloride additions was determined. In one series the effect was extended by adding potassium chloride to the original milk, thus increasing the range of total freezing-point depression.

Results are given in Table 2 and the relationship between chloride added and increase in freezing-point depression is shown in Fig. 2.

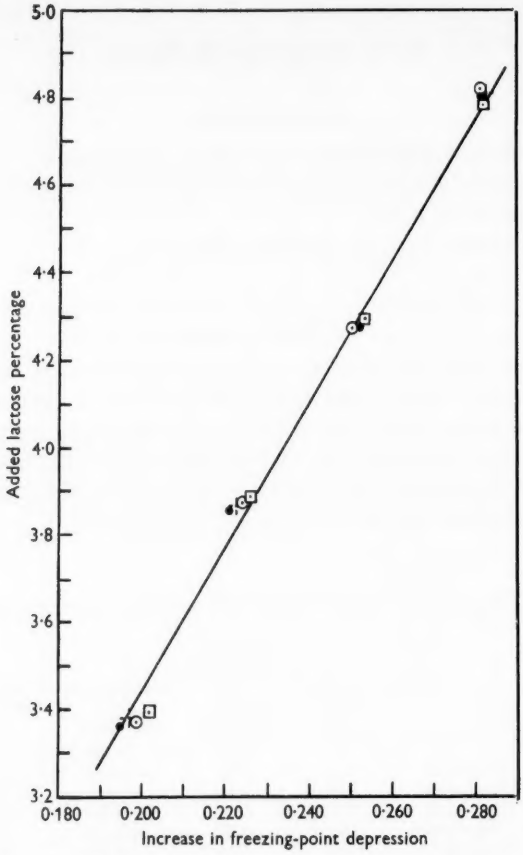

Fig. 1. Relationship between added lactose and increase in freezing point-depression. ⊙ relates to A series. • relates to B series. ⊡ relates to C series. + relates to D series.

Table 2. *Relationship between added chloride and increase in freezing-point depression*

Series	Sample	Potassium chloride added (g.)	Total Cl^- found (%)	Freezing-point (° C.) (Hortvet)	Increase in Cl^- (%)	Increase in freezing-point depression (° C.)
A	1. Original milk	—	0·113	−0·541	—	—
	2. 250 g. milk, 60 g. water	—	0·092	−0·432	—	—
	3. 250 g. milk, 60 g. water	0·2610	0·127	−0·474	0·035	0·042
	4. 250 g. milk, 60 g. water	0·5220	0·168	−0·515	0·076	0·083
	5. 250 g. milk, 60 g. water	0·7830	0·223	−0·571	0·131	0·139
	6. 250 g. milk, 60 g. water	0·9110	0·227	−0·581	0·135	0·149
B	1. Original milk	—	0·102	−0·537	—	—
	2. 210 g. milk, 60 g. water	—	0·078	−0·413	—	—
	3. 210 g. milk, 60 g. water	0·2610	0·118	−0·460	0·040	0·047
	4. 210 g. milk, 60 g. water	0·5220	0·164	−0·505	0·086	0·092
	5. 210 g. milk, 60 g. water	0·7830	0·207	−0·553	0·129	0·140
	6. 210 g. milk, 60 g. water	0·9110	0·233	−0·575	0·155	0·162
C	1. Original milk	—	0·086	−0·541	—	—
	2. 250 g. milk, 60 g. water	—	0·070	−0·434	—	—
	3. 250 g. milk, 60 g. water	0·2610	0·108	−0·474	0·038	0·040
	4. 250 g. milk, 60 g. water	0·5220	0·148	−0·515	0·078	0·081
	5. 250 g. milk, 60 g. water	0·7830	0·187	−0·559	0·117	0·125
	6. 250 g. milk, 60 g. water	1·0440	0·224	−0·597	0·154	0·163
D	1. Original milk	—	0·099	−0·542	—	—
	2. Original milk	0·2610	0·133	−0·586	0·034	0·044
	3. Original milk	0·5220	0·176	−0·628	0·077	0·086
	4. Original milk	0·7830	0·216	−0·668	0·117	0·126
	5. Original milk	1·0440	0·250	−0·711	0·151	0·169

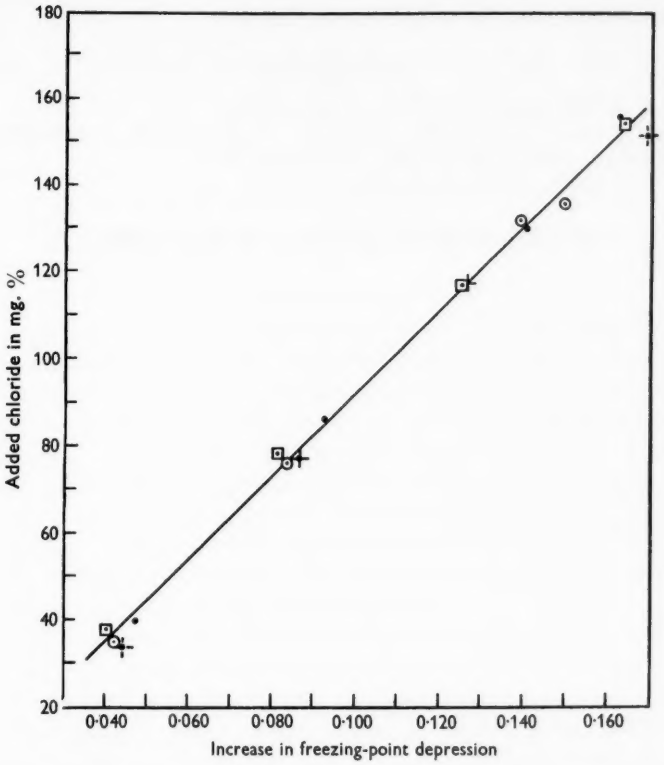

Fig. 2. Relationship between added chloride and increase in freezing-point depression. ⊙ relates to A series. • relates to B series. ⊡ relates to C series. + relates to D series.

DISCUSSION OF RESULTS

Under the conditions described, there is clearly a direct relationship between each of the components and their effect on the freezing-point depression, and this effect is constant over a range of concentrations. It seems probable that the same position obtains in the original milk samples, i.e. there is a definite and regular contribution by the lactose and chlorides according to their concentration.

From this work it is concluded that the freezing-point depression due to five per cent hydrated lactose is 0·294° C. and that due to 0·1 % chlorides (as chloride ion) is 0·110° C.

The result for lactose is in accordance with that indicated by Porcher & Chevallier (1923).

SUMMARY

The contributions of lactose and chloride to the total freezing-point depression of milk have been measured by an incremental procedure. Successive additions of these substances to watered milk have enabled direct determination of the partial freezing-point depression to be made in a medium substantially the same as the original.

Thanks are due to Mr J. V. Pascoe for his keen interest and to the Dairy Farmers' Co-operative Milk Co. Ltd. for co-operation in this work.

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637.147.128

600. FURTHER OBSERVATIONS ON THE VOLATILE COMPOUNDS ASSOCIATED WITH OXIDIZED FLAVOUR IN SKIM MILK

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INTRODUCTION

The addition of copper to skim milk in the range of 1–10 p.p.m. causes an off-flavour which has been described by various workers as metallic, papery, cardboard or, simply, oxidized (1). Pont (1,2) concluded that this flavour in skim milk was identical with the cardboard flavour component of copper-induced oxidized flavour in whole milk. The compounds causing cardboard flavour in milk are steam volatile and can be separated from affected milk by distillation under reduced pressure (1). Forss, Pont & Stark (3) identified acetone, acetaldehyde, *n*-hexanal, crotonaldehyde, and the C_5–C_{11} 2-unsaturated aldehydes (2-enals) in distillates from cardboard-flavoured skim milk. They concluded that the C_5–C_{11} 2-enals were important constituents of cardboard flavour with *n*-hexanal and crotonaldehyde possibly playing a subsidiary role.

Presumptive evidence for the presence of several 2,4-dienals in distillates from cardboard-flavoured milk was also obtained in this investigation (3). When tested in milk the flavour of 2,4-hexadienal strongly resembled cardboard flavour, and it was suspected that 2,4-dienals might also play an important part in the cardboard flavour defect. As the result of further investigation it can now be reported that the C_6–C_{11} 2,4-dienals have been identified as constituents of cardboard flavour in milk.

EXPERIMENTAL AND RESULTS

(*a*) *Methods of isolation and identification*

Skim milk with copper-induced cardboard flavour was distilled at 5 mm. mercury pressure in approximately 33 gal. quantities. The aqueous distillates from 100 gal. of milk were combined and concentrated by further distillation; the final concentrated distillate was then extracted with light petroleum. Carbonyl compounds (the cardboard flavour was found to be associated with the carbonyl fractions), including the 2,4-dienals, were isolated and identified in the form of their 2,4-dinitrophenylhydrazones. A detailed account of the procedures used is given in the previous paper by Forss *et al.* (3). No new distillations were carried out in the present instance; the isolations were made from material held over from the earlier investigation.

Fractions of mixed 2,4-dinitrophenylhydrazones were chromatographed on silica gel and/or alumina columns until constant R_f value by paper chromatography, ultra-violet absorption maximum (λ max.) and melting-point were attained. Identity was established by comparison of the isolated compounds with synthetic standards, particular regard being paid to the R_f value, λ max., melting-point and mixed melting-point.

(*b*) *Preparation of* 2,4-*dienals*

The synthesis of 2,4-hexadienal was described in the previous paper (3).

2,4-Heptadienal was prepared from propionaldehyde by the method of Forss & Hancox (4).*

2,4-Nonadienal was obtained from 2-heptenal by the method of Forss & Hancox (4). The 2-heptenal was prepared from heptanal by the method of Kuhn & Grundmann (5).

2,4-Undecadienal was prepared from 2-nonenal as described by Forss & Hancox (4). The 2-nonenal was obtained from castor oil according to Scanlan & Swern (6).

(*c*) *The* 2,4-*dienals isolated from cardboard-flavoured skim milk*

The 2,4-dienals isolated and the data on which their identity is based are shown in Table 1. The proportions of the individual compounds are estimates based on colour intensities in solutions and on chromatograms. Even with the most abundant compound, 2,4-heptadienal, only about 2 mg. of the 2,4-dinitrophenylhydrazone was available.

Table 1. *Identification of the* 2,4-*dinitrophenylhydrazones isolated from cardboard skim milk*

2,4-Dinitrophenyl-hydrazone	Colour	Percentage of 2,4-dienals	Melting-point (° C.)	Mixed melting-point (° C.)	R_f of compound with equivalent value	λ max. (mμ)
Isolated 2,4-hexadienal	Deep red	1	176–179	—	2,4-Hexadienal	389
Synthetic 2,4-hexadienal	Deep red		185–186			389
Isolated 2,4-heptadienal	Deep red	50	154–156	153–156	2,4-Heptadienal	389
Synthetic 2,4-heptadienal	Deep red		153–156			389
Isolated 2,4-octadienal	Deep red	1	152–153	—	Between 2,4-hepta-dienal and 2,4-nonadienal	389
Isolated 2,4-nonadienal	Deep red	30	135–137	135–138	2,4-Nonadienal	389
Synthetic 2,4-nonadienal	Deep red		136–138			390
Isolated 2,4-decadienal	Deep red	10	142–144	—	Between 2,4-nona-dienal and 2,4-undecadienal	389
Isolated 2,4-undecadienal	Deep red	5	131–134	131–133	2,4-undecadienal	389
Synthetic 2,4-undecadienal	Deep red		133–134			389

Other 2,4-*dinitrophenylhydrazones of compounds isolated from cardboard skim milk*

(i) Deep red crystals, m.p. 119–122° C. from light petroleum, R_f above 2-undecenal, λ max. 389 mμ. This may be the C_{14} 2,4-dienal and represents about 2% of the 2,4-dienals.

(ii) Yellow crystals, m.p. 216–218° C. from ether, R_f equal to two-thirds formaldehyde, λ max. 394 mμ.

(iii) Orange red crystals, m.p. 153–154° C. from light petroleum, R_f just below formaldehyde, λ max. 397 mμ and smaller peak at 430 mμ.

These two compounds do not appear to be 2,4-dienals. Compound (ii) may be a furfural derivative (cf. furfural, m.p. 223° C., R_f equal to two-thirds formaldehyde, λ max. 390 mμ (7, 8)). Compound (iii) may be a mixture of a furfural compound and a highly unsaturated compound.

* $R.CH{:}CH.CHO \xrightarrow[\text{Pyridine}]{\text{Malonic acid}} R.(CH{:}CH)_2COOH \xrightarrow[\text{Ether}]{LiAlH_4} R.(CH{:}CH)_2CH_2OH \xrightarrow[\text{Pet. ether}]{MnO_2} R.(CH{:}CH)_2CHO.$

(d) Flavour tests with 2-enals and 2,4-dienals in skim milk

The following unsaturated aldehydes isolated in this and the previous investigation were tested for flavour in skim milk: 2-octenal, 2-nonenal, 2,4-heptadienal, 2,4-nonadienal, 2,4-undecadienal. The flavour of 2-nonenal was examined also; this compound was not identified in distillates from milk, but when prepared in the synthesis of 2,4-undecadienal its odour was thought to strongly resemble that of oxidized flavour in milk.

The compounds were added to fresh skim milk in concentrations of 10^{-7}–10^{-9} and submitted with samples of skim milk containing natural copper-induced cardboard flavour to a grading panel. The artificial samples were adjusted to give as far as possible the same intensity of flavour as the natural cardboard milk samples. The grading panel consisted of from five to seven laboratory workers all of whom had had previous experience in grading milk with oxidized flavour. The identities of the samples were concealed under code numbers during grading.

The results of the flavour tests are summarized as follows.

(1) In seventy-five individual flavour judgements the natural cardboard-flavoured milk was described correctly in 93% of cases.

(2) In 111 flavour judgements on the samples containing the synthetic compounds, 20% were described as 'cardboard flavour' without any qualification. In 67% of cases the description indicated a close resemblance to cardboard flavour such as 'atypical cardboard' or 'cardboard' with various accessory descriptions such as 'drying-oil', 'painty', 'linoleum', 'varnish'. In the remaining 13% no clear resemblance to cardboard flavour was indicated; descriptions in this category included 'almond', 'carrots', 'yeast or beef-extract', 'aldehyde'.

(3) Resemblance of the synthetic compounds to natural cardboard flavour was more pronounced at the lower levels of flavour intensity. At the higher levels, when the qualification 'strong' was frequently used in the description, 28% of forty-six individual judgements indicated no resemblance. At the lower levels, with weak to medium intensity flavours only one out of sixty-five cases indicated no resemblance.

(4) There were no obvious differences between any of the six compounds in their resemblance to cardboard flavour. Several mixtures of 2-enals and 2,4-dienals with proportions based on their relative abundance in the distillates were flavour-tested also; none of these suggested a closer resemblance to cardboard flavour than the individual compounds.

DISCUSSION AND CONCLUSIONS

It was shown previously (3) that the C_5–C_{11} 2-enals are important constituents of cardboard flavour in milk. The evidence given here confirms the suggestion made in the earlier report that a number of 2,4-dienals are also characteristically associated with the defect. Both the mono- and di-unsaturated aldehydes, when added to milk, have a similar flavour closely resembling the defect with which they are associated, and it is concluded that together they constitute a basic and characteristic element of copper-induced cardboard flavour in milk. From their relative abundance in distillates from affected skim milk, 2-octenal, 2-nonenal, 2,4-heptadienal and 2,4-nonadienal appear to be the most important of the aldehydes.

Though the cardboard flavour was carried essentially by the carbonyl fraction during

the isolations, it was observed on occasions that a small part of the flavour was retained in the non-carbonyl fractions. It is possible that compounds closely related to the unsaturated aldehydes and possessing the same type of flavour like 2-nonenal could play a minor part in the defect in milk. In any case it is clear from the flavour tests that while these compounds in milk closely simulate cardboard flavour, the resemblance is not complete and that the defect contains further subsidiary flavour elements.

The probable mechanism for the production of short to medium chain length 2-enals and 2,4-dienals in milk is through the scission by autoxidation of a double bond in the more highly unsaturated fatty acids. It is of interest to observe that linolenic acid is now thought (9) to be the source of the 'grassy', 'painty' and similar off-flavours in reverted soybean and linseed oils. Furthermore, mono- and di-unsaturated aldehydes have been identified among the volatile compounds isolated from reverted soybean oil (10,11). Lea (12) has compared the development of off-flavours at low levels of oxidation in milk fat with the phenomenon of reversion in vegetable oils; the results from the present investigation would appear to support this analogy.

SUMMARY

The C_6–C_{11} 2,4-dienals have been identified in distillates from skim milk with oxidized flavour. The C_4–C_{11} 2-enals were isolated from the same distillates in a previous investigation. The most abundant individual compounds in the two groups were 2-octenal, 2-nonenal, 2-4-heptadienal and 2,4-nonadienal. These compounds when flavour-tested in skim milk in dilutions of 10^{-7}–10^{-9} closely resembled oxidized (cardboard) flavour. It is concluded that they are the main constituents of this flavour defect and that they originate from the oxidation of the more highly unsaturated fatty acids in milk lipids.

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601. THE EFFECT OF SALT AND OF ANTIOXIDANTS ON THE KEEPING QUALITY OF BUTTER

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Sweet-cream butter produced from good-quality cream is usually free from any major defect in flavour. During some months of holding at low temperature, however, it may lose its freshness of flavour and develop, to an extent varying widely for different types of butter, a measure of 'staleness' which in New Zealand is commonly termed 'storage' flavour. Barnicoat & Palmer (1) concluded that this storage flavour was due to an oxidation of the unsaturated acids of the triglycerides of butterfat but only to so slight a degree that it could not be detected by chemical analysis. Wiley (2) stated that there was no evidence to show whether storage flavour was caused by a slight oxidation, which could not be detected chemically or by some other form of deterioration in the butter.

Addition of antioxidants has been found effective in retarding oxidation of unsaturated acids in fats and oils, e.g. lard, thus preventing development of oxidized flavour defects. Butter differs from these fats and oils both in composition of the fatty portion and also because of its greater heterogeneity in composition and structure—it consists of butterfat globules, fat crystals, buttermilk droplets, water or brine droplets and air bubbles dispersed in a continuous phase of liquid butterfat. Nevertheless, some antioxidants* have been found useful in the control of flavour deterioration in acid-cream butters of European manufacture (3,4). If storage flavour is caused by an incipient fat oxidation, its development should be retarded by antioxidants, and it should be accelerated by pro-oxidants, such as salt (2,3). Experiments on the effect of antioxidants on the keeping quality of butter therefore could provide a useful index of the nature of the 'storage' flavour defect. The present paper describes an investigation of the effects of antioxidants of a number of types on the keeping quality of New Zealand sweet-cream butter.

EXPERIMENTAL

Manufacture of butter. The butters were made at the Institute experimental factory from fresh cream neutralized with sodium bicarbonate to an acidity of 0·07–0·09% lactic acid and pasteurized in a Vacreator† vacuum pasteurizer. The cooled cream (approximately 400 lb. per churning), after holding overnight, was churned and the butter was worked in an internal worker churn. To most of the butters, including all those treated with antioxidants, salt was added at the granule stage to give a final salt content of 1·6% in the butter.

* Addition of antioxidant substances to butter is illegal in New Zealand, and also in the United Kingdom, to which a large proportion of the annual output of New Zealand butter is exported. The additions reported here were for experimental purposes only.

† 'Vacreator' is the registered trade mark of the Murray vacuum pasteurizer manufactured by Murray Deodorizers Ltd., Auckland, New Zealand.

Addition of antioxidants. When antioxidants were used they were added either to the cream before churning or to the butter at the granule stage just before the addition of salt. Antioxidants were added in solution, or in an emulsion, or in the form of a paste with water.

Storage of butter. One 56 lb. box from each churning was held at 14° F. for 4 months and a separate box was held at this temperature for 8 months. In addition, however, for investigation of the keeping quality at atmospheric temperatures after storage, two 4 lb. boxes were packed from each churning. These were taken out of storage at 4 and 8 months after manufacture and were then held in the dark at 60–65° F. for 12–14 days before examination.

Grading of the butters. The butters were graded by professional butter graders when fresh, after 4 and 8 months' storage and after a further 2 weeks at 60–65° F.

Buttermilk butter. Because of its high phospholipid content the effect of storage on the flavour of butter from buttermilk cream is of considerable interest. Cream, separated from buttermilk at temperatures of 60–100° F., was churned in the usual manner (the time of churning was approximately twice that for the churning of fresh cream). The butter was of normal moisture content and of good quality.

Small-scale laboratory churnings. For some of the experimental work small quantities of butter were prepared in the laboratory from creams of different types. The creams were held overnight in the refrigerator and were churned in the apparatus described by Dolby (6).

'Washed' cream was prepared by separating fresh pasteurized cream diluted with water. Dilution and separation of the cream were repeated twice more. Artificial creams were prepared by mixing melted butterfat with various liquids heated to 50° C. and passing the mixture through a hand homogenizer three times. The liquids used included skim milk, whey, butter serum and water mixed with various emulsifying agents.

The butters from these creams were stored at 14° F. for 4 and 8 months and then were examined by graders at the Institute. Also small samples of butterfat and of serum, prepared from fresh butter, were stored at 14° F. and examined after storage.

Preparation of samples of butterfat and serum for analysis. A sample (approximately 70 g.) of butter in a 100 ml. centrifuge tube was melted at 40° C. The fat, clarified by centrifuging for a short time, was decanted and filtered at 40° C. When the butter serum was required for analysis the sample was centrifuged again, most of the remaining fat was drawn off, the tube was cooled in a refrigerator to solidify the last traces of fat, and the fat-free serum was poured off into a clean test-tube.

Fat oxidation and other analytical tests

(*a*) *Peroxide value.* The peroxide values of butterfats from fresh and stored butters were often too low to be estimated accurately by the Lea (1938) method (7). For peroxide values greater than 0·2 m.equiv. of oxygen per kg. of fat fairly reliable results were obtained by a modification of Lea's method (8). For all routine testing of butterfat, however, the thiocyanate method as described by Loftus Hills & Thiel (9) proved more satisfactory. The red thiocyanate colour was measured in a Spekker Absorptiometer with Ilford colour filter 604. A standard correction was made for the natural colour of the butterfat. All the peroxide values recorded in the tables were estimated by this method. They are quoted in milli-equivalents of oxygen per kg. of fat. A comparison of the results obtained by the

modified Lea method and by the thiocyanate method on a large number of butterfats (Lea peroxide values 0·2 and higher) showed that the thiocyanate value on the average was 1·5 times that of the Lea value.

(*b*) *Fat aldehyde values.* These were determined according to the method of Schibsted (10), but with use of sodium metabisulphite for preparation of the reagent (11) and of the Spekker Absorptiometer with Ilford colour filter 604 for measurement of the colour intensity (12). The fat aldehyde values of the fresh butterfats were all under 0·1. Hence they are not quoted in the tables.

(*c*) *Thiobarbituric acid (TBA) test.* The TBA test, based, presumably, on the reaction between TBA and malonic dialdehyde (13), has been applied to milk (14) and to butterfat (13). In the present investigation it was found that little, if any, colour developed in the test with butterfat even from stored butter; but definite colour was obtained with serum from fresh butter and a markedly increased intensity of colour with serum from stored butter. The procedure for the TBA test in the serum was as follows:

'To 2·5 ml. of serum, separated as described above, add 2·5 ml. of water and 2·5 ml. of TBA reagent. Heat in a boiling water-bath for 10 min., cool and extract with 5 ml. of 2 : 1 isoamyl alcohol/pyridine mixture. Centrifuge for 10 min., pour off the extract, filter and measure the colour intensity in the Beckman Spectrophotometer, at 535 mμ. Express the results as the optical density of the extract on this wavelength.'

The fairly high values for fresh butter serum apparently are not due solely to oxidized fat but are, in part, caused by some other compound in the serum reacting with TBA. There is no explanation for the unusually high values for the serum from fresh unsalted butter (see Table 1), since the addition of salt to the serum has little or no effect on the final reading.

(*d*) *Induction period test.* The induction periods of butterfats from butters treated with fat-soluble antioxidants were measured by means of the Swift stability test, with dried air. The end-point was taken as the time when, owing to oxidation of the carotene, the butterfat had almost completely lost its yellow colour.

(*e*) *Acid values of the butterfat.* These were measured by the A.O.A.C. method (15). Since, with the exception of the butterfat from one unsalted butter, there was no increase in fat acidity during storage the fat acidity results are not shown in the tables.

(*f*) *Oxidation-reduction potential of butter serum.* Oxidation-reduction potentials were measured with a Beckman pH meter, using a calomel half-cell connected by an agar/potassium chloride glass bridge to the serum in a small glass beaker. Platinum wires were used as electrodes, one end in the form of a coil dipping into the serum and the other end connected directly to the pH meter by means of a spring clip and copper wire. Two electrodes were left immersed in each butter serum sample for 15 min. before readings were taken on the pH meter. They were cleaned in boiling concentrated nitric acid after use and were stored in distilled water.

(*g*) *Ascorbic acid content of butter serum.* The loss of ascorbic acid which occurs during the preparation of the serum from butter has been referred to by Allen (16). The following method reduces this loss to approximately 5%:

'Add 25 ml. of chloroform and 8 ml. of 10% metaphosphoric acid to 25 g. of butter and heat over a small flame until the butterfat is dissolved. Transfer to a 100 ml. centrifuge tube and centrifuge. Titrate directly with 2:6 dichloro-phenol-indophenol into the aqueous layer, stirring gently with a glass rod.'

For the routine estimation of added ascorbic acid in salted butter it was found satisfactory to prepare the serum by melting the butter at 40° C. After centrifuging, as much fat as possible was drawn off and the serum was quickly cooled in an ice-bath, well shaken, and poured off from any remaining fat. An accurately measured volume of serum (5 ml. for control butters, 2 ml. for butters with added ascorbic acid) was acidified with sulphuric acid and titrated with 0·04% 2:6 dichloro-phenol-indophenol according to the method of Sharp for milk (17). The remainder of the serum was then added for the measurement of Eh value. This method of preparation gave up to 10–15% loss of ascorbic acid, but it was quite satisfactory for comparative purposes.

RESULTS

Effect of salt

The addition of salt to emulsions of butterfat and water or skim milk has a definite pro-oxidant effect on the butterfat (5). Earlier work at this Institute had shown already that with the analytical tests then employed there was no evidence of fat oxidation after storage of unsalted butter (2,18). Also it appeared to be generally accepted that unsalted butter was less liable to develop storage flavour than salted butter.

In the present investigation the average results of five comparisons over the period September to January inclusive, of unsalted butters with salted butters containing 1·6 and 2·5% of salt made from batches of the same bulk supply of cream, are given in Table 1. The results for the fat oxidation show that butterfat oxidation does occur in unsalted butters during storage but to a lesser extent than in salted butter. Increasing the salt content from 1·6 to 2·5% decreased the extent of fat oxidation in salted butter to little more than that of unsalted butter. There was no appreciable difference in intensity of storage flavour after 4 and 8 months between the unsalted butter and the butter

Table 1. *Effect of salt on the keeping quality of butter and on the oxidation of the butterfat*

	Time of storage				
	14° F. months	65° F. weeks	(1) Unsalted	(2) 1·6% salt	(3) 2·5% salt
Peroxide value	0	—	0·09	0·10	0·09
	4	—	0·11	0·21	0·12
	8	—	0·22	0·56	0·30
	8	2	0·43	0·98	0·55
Aldehyde value	4	—	0·20	0·24	0·20
	8	—	0·25	0·79	0·31
	8	2	0·39	1·38	0·59
TBA test	0	—	0·44	0·25	0·22
	4	—	0·61	0·51	0·39
	8	—	0·65	0·62	0·47
	8	2	0·74	0·95	0·67
Average grade score and remarks	0	—	92·9 (fair quality)	92·4 (fair quality)	91·7 (harsh)
	4	—	92·5 (storage)	92·0 (storage)	90·8 (harsh)
	8	—	92·2 (storage)	91·7 (storage)	91·0 (less storage but harsh)
	8	2	88·0 (tallowy)	89·0 (strong storage or tallowy or fishy)	90·4 (storage and saline)

containing 1·6% salt. Butter containing 2·5% salt was reported as having less storage flavour, but it was graded lowest after 8 months' storage because of the 'harshness' resulting from the high salt content.

Holding of the butter at 60–65° F. after 8 months' cold storage reduced the grade score of the highly salted butter by only 0·6 point but lowered the grade of the unsalted and the lightly salted butters by 3–4 points, bringing them into the second grade class. The fall in grade score was due to development of strong storage and tallowy flavours. The oxidation values of the butterfats from the butters after storage, cited in Table 1, do not show any relationship to the type or intensity of the flavour deterioration reported by the butter graders.

Effect of antioxidants

(1) *Fat-soluble antioxidants*

(*a*) *Nordihydroguaiaretic acid* (*NDGA*). This substance has been found to prevent flavour deterioration in a number of commercial fats (19), and to retard development of objectionable oxidized flavours in a variety of dairy products (20,21). In butter, however, the results have been less promising (4, 22).

In trials at this Institute NDGA was added, in the form of a paste with water, to either the cream or butter in the proportion of 0·005% of the expected finished butter, along with citric acid in the same proportions. The results in Table 2, which are the averages for nine separate trials, show that this antioxidant greatly reduced the extent of fat oxidation occurring during storage over 4 and 8 months; but storage flavour was evident in both the treated and the untreated butters after 8 months. In the treated butters also there were 'slight chemical or metallic flavours' or 'unattractive after-flavours' which presumably were due to the NDGA itself or to its breakdown product, and which caused a slight lowering of the grade score in comparison with the controls.

Table 2. *Effect of NDGA on the keeping quality of butter and on the oxidation of the butterfat*

	Time of storage				
	14° F. months	65° F. weeks	(1) Control	(2) Addition of NDGA (0·005% of expected butter) to cream	(3) Addition of NDGA (0·005%) to butter
Peroxide value	0	—	0·07	0·07	0·06
	4	—	0·17	0·11	0·07
	8	—	0·46	0·15	0·13
	8	2	0·70	0·23	0·22
Aldehyde value	4	—	0·24	0·20	0·20
	8	—	0·44	0·21	0·20
	8	2	1·02	0·23	0·23
Induction period	8	—	12 hr.	27 hr.	31 hr.
Average grade score and remarks	0	—	93·0	92·9	92·9
	4	—	92·7 (little or no storage)	92·4 (trace of chemical or metallic flavour)	92·2 (trace of chemical or metallic flavour)
	8	—	92·0 (storage)	91·7 (storage and unattractive after-flavour)	91·6 (storage and unattractive after-flavour)
	8	2	89·6 (strong storage)	90·3 (less storage than (1) but harsh or chemical flavour)	90·1 (less storage than (1) but harsh or chemical flavour)

Addition of citrates has been found by Barnicoat (18) to have no effect on the quality of butter, and in the present investigation addition of citric acid in the proportions reported above did not have any significant effect on the antioxidant properties of NDGA in sweet-cream butter.

The induction period results for the butterfat, given in Table 2, show that, after 8 months, NDGA was still effective as an antioxidant.

(*b*) *Octyl and dodecyl gallates.* The fat-soluble esters of gallic acid with the higher alcohols have been found to be effective antioxidants in lard (23), in beef tallow (24) and in butterfat and dried milk (25). When added to acid-cream butters before storage at 12–15° C. they did not give any improvement in keeping quality although they greatly retarded oxidation of the butterfat (25).

Two trials were made with octyl gallate and two with dodecyl gallate, added as an emulsion in water to cream or to butter. The results are given in Table 3. Both the gallates retarded oxidation in the butterfat, but they did not prevent the onset of storage flavour during 8 months' cold storage. After a further 2 weeks at 60–65° F. there was considerably less storage flavour in the gallate-treated butters than in the control butter.

The induction period results for the butterfats from the stored butters show that the gallate esters retained their antioxidant properties over the 8 months' storage period. To save space the results for oxidation of the butterfats and for the grading scores of the butters after 4 months' storage have been omitted from Table 3 and subsequent

Table 3. *Effect of octyl and of dodecyl gallates on the keeping quality of butter and on the oxidation of the butterfat*

	Time of storage				
	14° F. months	65° F. weeks	(1) Control	(2) Addition of gallate (0·01 %) to butter	(3) Addition of gallate (0·002–0·005 %) to butter
			Octyl gallate		
Peroxide value	0	—	0·18	0·14	0·18
	8	—	0·42	0·19	0·23
	8	2	0·79	0·30	0·29
Aldehyde value	8	—	0·43	0·20	0·27
	8	2	1·12	0·29	0·35
Induction period	8	—	10 hr.	54 hr.	45 hr.
Average grade score and remarks	0	—			
	8	—	92·0 (storage)	91·6 (storage and slight foreign flavour)	91·5 (more storage than (1))
	8	2	91·0 (strong storage)	91·5 (less storage than (1))	91·5 (less storage than (1))
			Dodecyl gallate		
Peroxide value	0	—	0·12	—	0·13
	8	—	0·35	—	0·16
	8	2	0·70	—	0·31
Aldehyde value	8	—	0·33	—	0·20
	8	2	1·20	—	0·36
Induction period	8	—	11 hr.	—	36 hr.
Average grade score and remarks	0	—			
	8	—	91·5 (storage)	—	91·5 (storage and slight foreign flavour)
	8	2	90·5 (strong storage)	—	90·0 (slightly less storage than (1), but unsound flavour)

tables. Fat oxidation results were intermediate between those for the butterfats from fresh and those from 8-month-old butters. Storage flavour was usually of only slight intensity at the 4 months' grading, and the effect of the additives was not easily discernible organoleptically. There was a marked increase in intensity of storage flavour and in oxidation values when the butters were kept at 60–65° F. for 2 weeks, but the effect of this extra period of storage was similar to that given in the tables after 8 months.

(2) *Water-soluble antioxidants*

(*a*) *Ascorbic acid.* The effect of added ascorbic acid on the keeping quality of dairy products has been extensively studied (26). Ascorbic acid retards the onset of 'oxidized' flavour in milk and increases the storage life of milk powder (26,27). Addition of ascorbic acid to sweet cream for butter manufacture was found by Barnicoat & Palmer (1) to have little effect on the keeping quality of butter at 40° F.; but with acid-cream butters Mulder, Kleikamp & King (28) reported that it prevented the development of 'metallic' or 'metallic-oily' defects. Addition of 0·01 % of ascorbic acid to frozen cream was found by Smith, Loewenstein, Anderson & Olson (29) to prevent development of 'oxidized' flavour, but the addition of 0·02 % to butter caused more pronounced 'oxidized' flavour than in the control butters. Tollenaar (3) found that addition of 0·02 % to butter stored at −8° C. prevented peroxide formation in the butterfat, but it did not retard the development of a 'train-oily' defect. Addition of larger quantities—0·05–0·2 %—to butter was found by Sabalitschka to prevent tallowy and other off-flavours at storage temperatures of 5–12° C., but it gave an objectionable malt-like flavour to the butter (30).

In the present investigation ascorbic acid was added in proportions ranging from 0·004 to 0·05 % for cream and from 0·001 % to 0·2 % for butter. The addition of 0·1–0·2 % to butter ensured that a relatively high concentration remained after storage of the butter, but these high concentrations lowered the pH to below 5·0; and although they prevented fat oxidation they caused such a strong 'coconut' flavour as to render the butter commercially unacceptable.

Some results of trials in which smaller proportions than those quoted above were added are given in Tables 4 and 5. The small amount of natural ascorbic acid in the butter soon disappears through oxidation (16); and most of the added ascorbic acid is lost during storage causing an increase in Eh values. Even in butters with only 0·001 % of added ascorbic acid there was little fat oxidation during storage; but at this and at higher concentrations there was no effect on the development of storage flavour (see Tables 4 and 5).

When the butters were held for a further 2 weeks at 60–65° F. the added ascorbic acid appeared to have a retarding effect on the further development of storage flavour.

Both with the control butters and with experimental butters churned from cream containing added ascorbic acid the ascorbic acid content of the fresh butter serum was found to be less than half that of the buttermilk. Part of this difference would be due to the presence of a considerable amount of wash water in the serum (31) and part to the 10–15 % loss of ascorbic acid in the preparation of the butter serum (see above); and where the analysis of the butter is delayed for some hours after churning part of the difference could also be due to the rapid destruction of ascorbic acid which occurs in the butter at

atmospheric temperatures as a result of the pro-oxidative catalytic effect of the small amount of copper present. The decrease in ascorbic acid content and the increase in copper content (16) would account also for the increase in Eh value of the butter serum in comparison with that of the buttermilk.

(*b*) *Alkali-treated skim milk.* The production of reducing substances in whey and skim milk by alkali treatment was reported by Sjöström & Larsson (32). They found that addition

Table 4. *Effect of added ascorbic acid on the keeping quality of butter and on the oxidation of the butterfat*

	Time of storage				
	14° F. months	65° F. weeks	(1) Control	(2) Addition of ascorbic acid (0·05%) to cream	(3) Addition of ascorbic acid (0·01%) to butter
Peroxide value	0	—	0·26*	0·27*	0·30*
	8	—	0·36	0·12	0·12
	8	2	0·66	0·16	0·26
Aldehyde value	8	—	0·29	0·20	0·20
	8	2	0·73	0·24	0·20
Ascorbic acid content of butter (mg./100 g.)	0	—	0·04	6·42	10·10
	8	—	nil	2·42	1·29
	8	2	nil	0·51	1·22
Oxidation-reduction potential of butter serum (volts)	0	—	0·340	0·240	0·226
	8	—	0·390	0·257	0·294
	8	2	0·393	0·319	0·293
Average grade score and remarks	0	—	92·5	92·3 (little harsh)	92·0 (least preferred)
	8	—	91·5 (storage)	90·0 (storage and foreign flavour)	90·0 (storage and foreign flavour)
	8	2	91·0 (storage)	91·5 (less storage than (1), slight chemical flavour)	91·5 (less storage than (1), slight chemical flavour)

* High initial peroxide values which may decrease on storage occur with certain butters.

Table 5. *Effect of very small quantities of added ascorbic acid on the keeping quality of butter and on the oxidation of the butterfat*

	Time of storage				
	14° F. months	65° F. weeks	(1) Control	(2) Addition of ascorbic acid (0·004%) to cream	(3) Addition of ascorbic acid (0·001%) to butter
Peroxide value	0	—	0·12	0·14	0·15
	8	0	0·12	0·10	0·07
	8	2	0·39	0·16	0·12
Aldehyde value	8	—	0·20	0·20	0·20
	8	2	0·38	0·24	0·20
Ascorbic acid content of butter (mg./100 g.)	0	—	0·09	0·52	0·78
	8	—	nil	0·04	0·18
	8	2	nil	nil	nil
Oxidation-reduction potential of butter serum (volts)	0	—	0·363	0·298	0·283
	8	—	0·329	0·351	0·342
	8	2	0·419	0·425	0·412
Average grade score and remarks	0	—	93·0	93·0	93·0
	8	—	92·5 (slight storage)	92·0 (storage and slight coarseness)	92·3 (slight coarseness)
	8	2	91·5 (strong storage)	91·8 (slightly less storage than (1))	92·0 (slightly less storage than (2))

of alkali-treated whey to cream before churning controlled oxidation and oxidized flavours in butter stored at 18° C.

In the present investigation, skim milk and whey, treated according to the directions of the above authors, were found by the method of Crowe, Jenness & Coulter (33) to have a high content of reducing groups. Since skim milk was more readily available than whey, and since it also had a higher content of reducing substances after treatment, it was used in the experimental work with cream and butter. The alkali-treated skim milk was added to give 2% by weight in the cream and 1% in the butter. The butter containing the additive was, when fresh, much darker in colour than the control, and it had a harsh 'caramel' flavour. The foreign flavour gradually disappeared during storage, but the colour remained noticeably darker than that of the control. After 8 months' storage there was no difference in intensity of storage flavour, but after a further 2 weeks at 60–65° F. the butters containing treated skim milk showed less fat oxidation and a less intense storage flavour than the control butters.

(*c*) *Citraconic acid.* This compound in concentrations of 0·03–0·045% has been reported to be a useful and harmless antioxidant in commercial margarine (34). In the present investigation 0·005–0·01% of citraconic acid in butter was found to retard fat oxidation only slightly; and it did not inhibit the development of storage flavour.

(3) *Surface-active agents*

Some surface-active agents, when added to whole milk powder, were reported by Busch, Decker & Ashworth (35) to have antioxidant properties, which were thought to indicate the formation of a surface adsorption film on the exposed surface of the fat particles. Loftus Hills & Conochie (5) suggested that these compounds could have a blanketing protective effect on the fat surface in butter and so retard fat oxidation.

Three surface-active agents, viz. Span 60, MYRJ 45 and Tween 60, have been investigated at this Institute. They were added, as a dispersion in water or as a solution in water to give concentrations of 0·02%, to the cream just before churning or to the butter at salting. They had no effect on the churning of the cream. Since the oxidation values for the butterfats and the grading results for the butters were similar for all butters containing the same proportion of surface-active agent, and irrespective of the agent itself, the results have been averaged (see Table 6). The additions had no effect on the flavour of the fresh butters and they had little effect in inhibiting flavour deterioration in the stored butters; but they all retarded oxidation in the butterfat. Since by virtue of their composition they are unlikely to have true antioxidant properties, it seems probable that they exerted a protective effect on the butterfat surface.

(4) *Compounds forming complex salts with copper*

The catalytic effect of traces of copper in increasing the rate of oxidation of butterfat in dairy products is well known. Observations at this Institute indicate that copper in sweet-cream butter in proportions greater than 0·25–0·30 p.p.m. will cause development of 'oily' or 'fishy' flavours during cold storage (see also (36)). Even smaller proportions than 0·25 p.p.m. were considered by Barnicoat & Palmer (1) to induce off-flavours, including 'stale-storage' flavour, in butter.

Addition of copper-complexing compounds has been found to inhibit the action of copper in solution with ascorbic acid (37); and to control or prevent the autoxidation of linoleic acid in buffered aqueous suspensions (38). Addition of similar compounds to dairy products, including butter, has been studied by a number of workers. In the present investigation some of these substances have been added to the cream before churning and to butter during manufacture, and their effect on the keeping quality of the butter has been studied.

(*a*) *Cysteine hydrochloride.* This substance has been found to inhibit oxidation of phospholipid and protein in dried whole-egg powder (39), but it did not prevent development of 'train-oily' taints in acid-cream butters (3). In a water-lard system cysteine has been reported (40) to have a pro-oxidant effect which was attributed to the absence of a phenolic antioxidant in the fat.

In the experimental butters at this Institute the presence of cysteine hydrochloride in butter in proportions as low as 0·005% caused an increase in the extent of oxidation of the butterfat (see Table 6), but had no influence on the intensity of storage flavour in the butters after 8 months at 14° F.

(*b*) *Di-sodium salt of ethylene diamine tetra-acetic acid* (*EDTA*). EDTA in aqueous solution in contact with lard has been found to retard fat oxidation (41). In the present investigation, however, when added to cream or to butter in proportions as low as 0·005 and 0·001% respectively, it, like cysteine hydrochloride (see above), accelerated fat oxidation (see Table 7). There was no effect on the flavour of the butter. Unlike NaDEDC (see below) it did not cause any transfer of copper from the butter serum to the fat.

Table 6. *Effect of surface-active agents on the keeping quality of butter and on the oxidation of the butterfat*

	Time of storage				
	14° F. months	65° F. weeks	(1) Control	(2) Addition of surface-active agents (0·02%) to cream	(3) Addition of surface-active agents (0·02%) to butter
Peroxide value	0	—	0·04	0·06	0·07
	8	—	0·67	0·46	0·36
	8	2	0·87	0·54	0·42
Aldehyde value	8	—	0·58	0·37	0·27
	8	2	1·36	0·56	0·32
TBA test	0	—	0·29	0·27	0·29
	8	—	0·67	0·46	0·41
	8	2	0·92	0·62	0·57
Average grade score and remarks	0	—	93·0	93·0	93·0
	8	—	92·3	92·2	92·0
	8	2	89·7 (strong storage or tallowy)	89·8 (slightly less storage than (1))	90·2 (less storage than (1))

(*c*) *Tetramethyl- and tetraethyl thiuram disulphide* (*TMTD and TETD*). In Holland it has been found that these compounds in proportions as low as 0·005% prevented the development of 'train-oily' flavours in acid-cream butters (3,4).

In the sweet-cream butters to which they were added in the trials at this Institute they had no effect on the intensity of storage flavour and, in addition, they caused development of 'chemical' or 'rancid' flavours during the 8 months' storage period. The fat oxidation results given in Table 8, which are typical for butters containing TMTD or TETD, show

that both these substances are effective antioxidants. The induction periods of the butterfats from the treated butters were greatly reduced, apparently because of the transfer of copper* from the serum to the fat (see also below under (*d*)). At the boiling-water temperature of the induction period test the copper may no longer be held as a complex in the fat and so may be free to act as a pro-oxidant.

Table 7. *Effect of the addition of cysteine hydrochloride and of EDTA to butter on the oxidation of the butterfat*

Substance added		Time of storage at 14° F. months	(1) Control	(2) Addition of substance to cream or butter	(3) Addition of substance to butter
Cysteine hydrochloride	—	—	—	0·01% to butter	0·02%
	Peroxide value	0	0·22	0·26	0·27
		8	0·46	1·33	1·33
	Aldehyde value	8	0·28	1·11	1·07
	TBA test	8	0·50	0·84	1·04
EDTA	—	—	—	0·01% to cream	0·003%
	Peroxide value	0	0·09	0·09	0·15
		8	0·19	0·72	0·80
	Aldehyde value	8	0·24	0·86	1·01
	TBA test	8	0·48	0·53	0·44

Table 8. *Effect of TETD on the keeping quality of butter and on the oxidation of the butterfat*

	Time of storage				
	14° F. months	65° F. weeks	(1) Control	(2) Addition of TETD (0·002%) to butter	(3) Addition of TETD (0·005%) to butter
Peroxide value	0	—	0·07	0·09	0·10
	8	—	0·19	0·15	0·16
	8	2	0·34	0·14	0·07
Aldehyde value	8	—	0·20	0·20	0·20
	8	2	0·36	0·24	0·24
Average grade score	0	—	92·5	92·5	92·5
and remarks	8	—	92·5 (storage)	91·5 (storage same as (1), chemical flavour)	91·5 (storage same as (1), chemical flavour
	8	2	91·0 (strong storage)	89·0 (rancid)	90·0 (slight storage, slight rancidity)

(*d*) *Sodium diethyl dithiocarbamate* (*NaDEDC*). Tollenaar(3) concluded that NaDEDC retarded development of 'train-oily' flavour in acid-cream butters. He found that it and also TMTD and TETD caused transfer of nearly all the copper from the butter serum to the butterfat in the melted butter. He has concluded that the antioxidant effect of these compounds is due to the absence of catalyzing copper in the fat-globule adsorption layer, with the result that the unsaturated acids in the phospholipids or the glycerides of the adsorption layer remain unoxidized. Also, because this primary oxidation no longer occurs, secondary oxidation in the true butterfat is not initiated and so peroxide values are low after some months of storage of the butter.

In the present investigation it was found that 0·005% of added NaDEDC in butter caused the appearance of 'foreign' flavours during storage. In the stored butters after

* The copper content of these butters was not determined.

a further 2 weeks at 60–65° F., these 'foreign' flavours were not so noticeable, and storage flavour then predominated (see Table 9). When these butters were melted approximately two-thirds of the copper was found in the butterfat (Table 9). The low values for fat oxidation in the treated butters even after 8 months' storage may be due to the reduced copper content of the serum or they may be due to an antioxidant effect on the butterfat of the copper diethyl dithiocarbamate itself at low temperatures.

Table 9. *Effect of NaDEDC on the keeping quality of butter and on the oxidation of the butterfat*

	Time of storage				
	14° F. months	65° F. weeks	(1) Control	(2) Addition of NaDEDC (0·01%) to cream	(3) Addition of NaDEDC (0·005%) to butter
Peroxide value	0	—	0·14	0·06	0·09
	8	—	0·29	0·18	0·15
	8	2	0·54	0·15	0·12
Aldehyde value	8	—	0·20	0·20	0·20
	8	2	0·45	0·20	0·20
TBA test	0	—	0·29	0·19	0·23
	8	—	0·41	0·28	0·32
	8	2	0·70	0·25	0·26
Copper content of serum (as p.p.m. in butter)	0	—	0·20	0·12	0·10
Copper content of fat (as p.p.m. in butter)	0	—	0·02	0·19	0·12
Average grade score and remarks	0	—	93·0	93·0	93·0
	8	—	92·3	91·5 (foreign flavour)	91·8 (less foreign flavour than (2))
	8	2	88·0 (rancid)	90·0 (strong storage flavour)	90·5 (less storage flavour than (2), foreign flavour)

Buttermilk butter

Butterfat oxidation in buttermilk butter after storage was greater than in normal butter although the intensity of storage flavour was similar. The phospholipid content of buttermilk butter was 3–4 times as great as that of normal butter.

Small-scale laboratory churnings

The flavour deterioration during storage of butters churned from artificial creams was seldom of the 'stale storage' type. Butter churned from washed cream, however, was graded 'strong storage' after 5 months at 14° F.

Storage flavour was found, on occasions, in stored butterfat and once in a butter reconstituted from fresh butterfat and stored serum. In separating the butter serum before storage, however, it is probable that traces of butterfat were included so that the storage flavour may have originated in the residual butterfat of the stored serum and not in the true serum constituents.

DISCUSSION

It has been shown that a 'stale-storage' defect occurs in both salted and unsalted butters and also in salted butters treated with antioxidants. This storage flavour may be caused by the formation of a single specific off-flavouring substance or it may be a composite flavour resulting from the formation in the butter of two or more substances with taste and odour. There are three possible sources of such substances, viz. (1) the butter serum, (2) the phospholipids and (3) the butterfat.

(1) *The serum.* Growth of bacteria in the butter serum can be a source of off-flavour in butter. In well-made sweet-cream butter, however, there is no increase in bacterial count during frozen storage. The flavour conceivably could be caused by a 'Maillard' or protein-sugar reaction between the casein and the lactose in the serum, analogous to the reaction causing 'stale' or 'gluey' flavours in dried skim milk (42); but at the small concentrations of lactose and protein in the serum, and at the low temperature of butter storage, occurrence of a Maillard reaction is unlikely. This conclusion is confirmed by the finding that storage flavour can develop in butter from 'washed' cream. The indications are, therefore, that storage flavour does not originate in the butter serum.

(2) *The phospholipids.* The breakdown of the lecithin molecule with the production of trimethylamine is no longer thought to be the cause of a 'fishy' flavour in acid butters (43, 44). Nevertheless, certain defects in stored butters are still held to be due to chemical reactions involving the phospholipids. Van der Waarden (45) found that in artificial mixtures of butter constituents only those butters with added phospholipids developed a storage defect typical of cold-stored butter. Van Haeften & Pette (4) considered that 'fishy' flavour was due to some form of lecithin deterioration in butter. Tollenaar (3) suggested that oxidation of the phospholipids of the fat-globule membrane was responsible for flavour deterioration in acid butter. It must be remembered, however, that with the reduction in proportion of globular fat from 80 to 18–28 % during working of the butter (46), the greater proportion of the phospholipids is no longer present as part of the fat-globule adsorption layer.

There is no evidence in the present investigation of the occurrence of a breakdown of the phospholipids of butter after long storage. Oxidation of the unsaturated acids of the phospholipids with subsequent decomposition to give an off-flavouring substance conceivably could take place, however, without affecting the estimated phospholipid content* of the butter. Indirect evidence that the phospholipids, or at least those not a part of the actual fat-globule membrane, are not responsible for the production of storage flavour is found in the flavour deterioration of buttermilk butter and of normal butter. The buttermilk butter, with a phospholipid content three or four times as great as that of normal butter, showed no significant difference in intensity of storage flavour compared with the normal butter.

Although the proportions of the fat of butter present in the globule form is so small, the phospholipids in the fat-globule membrane surrounding the fat cannot be overlooked as a possible source of the off-flavouring substance. Oxidation defects in the membrane may be promoted by the copper bound in the phospholipids-protein complex (44); and this copper may not react with substances such as NaDEDC, thus explaining the production of

* The phospholipid content of butter was estimated from the phosphorus content of a Röse-Gottlieb extract of the butter serum.

storage flavour in the presence of copper-complexing compounds. There is, however, no experimental evidence of the occurrence of such an oxidation nor of a defect in flavour arising therefrom.

(3) *The butterfat.* The occasional appearance of storage flavour in dry butterfats stored at low temperatures and the effect of antioxidants in inhibiting further increases in intensity of storage flavour after the butters are placed at 60–65° F. support the theory of Barnicoat & Palmer (1) that storage flavour is due to oxidation of the unsaturated acids of the glycerides of the butterfat. Butterfat oxidation values for butters after storage, however, varied greatly with changes in the condition of manufacture of the butter. Values for unsalted butter were much lower than for salted butter with a normal salt content of 1·6%.* The addition of either water-soluble or fat-soluble antioxidants or of certain copper-complexing compounds to salted butter also retarded butterfat oxidation very effectively. Oxidation in butter may originate in the butterfat itself (Loftus Hills & Conochie (5)) and further oxidation in the fat would be retarded by fat-soluble antioxidants. If peroxide groups from oxidation of the fat are present in the fat-serum interface, then salt should promote and water-soluble antioxidants and copper-complexing compounds should retard further oxidation. Alternatively, if oxidation begins in the serum (Van der Waarden (44)) and is transferred to the fat by means of the serum-fat interface, then both the pro-oxidant effect of salt and the antioxidant effect of water-soluble and fat-soluble antioxidants again can be explained.

Although in the present investigation butterfat oxidation was considerably less in unsalted butters and in salted butters treated with antioxidants than in untreated salted butters (of normal salt content), the intensity of storage flavour was similar in all butters from the same bulk cream. It appears that the extent of fat oxidation, as measured by the routine chemical tests, gives no estimate of the extent of the reaction involving the production of the causative substance of storage flavour.

Flavour deterioration in a refined fat or oil may take place on very slight oxidation and long before the appearance of true oxidative rancidity (47). This 'flavour reversion', which is not affected by added antioxidants, is probably related to oxidation in the glycerides containing highly unsaturated fatty acids (47, 24). Acids of this type already have been found in New Zealand butterfat (48, 49). In stored butterfat and butter, oxidation of these highly unsaturated acids may be the cause of storage flavour. Although this slight oxidation may not be detected by chemical tests, oxidation, during storage, of the less unsaturated acids also may occur, depending on the presence of salt and of antioxidants. Thus the apparent anomaly of butters with the same intensity of storage flavour but varying widely in butterfat oxidation values may be explained. When the butters are placed at higher temperatures (60–65° F.) after storage, antioxidants may then be effective in retarding further oxidation both of the highly unsaturated acids and of the less unsaturated acids. Under these conditions both the oxidation value of the butterfat and the intensity of storage flavour of the butter will increase only slowly.

While no direct evidence of the source or the nature of the off-flavouring substance has been brought forward in the present paper, it is established that onset of storage flavour in sweet-cream butters after long storage is not prevented by the addition of antioxidants.

* The decrease in the extent of fat oxidation on increasing the proportion of salt from 1·6 to 2·5% is surprising and apparently has not been noted previously.

SUMMARY

Butterfat oxidation occurred in both salted and unsalted butters during storage at 14° F.

Butterfat oxidation values, after storage, were much lower in unsalted and in highly salted butters than in butters of normal salt content.

Butterfat oxidation in salted butters during storage was retarded by treatment with antioxidants. These included fat-soluble and water-soluble substances, surface-active agents and copper-complexing compounds.

Unsalted butter, salted butter and salted butter treated with antioxidants all developed 'storage' flavour during storage for eight months.

Many of the antioxidants added to salted butter were effective in retarding further fat oxidation and further increase in intensity of storage flavour when the butters were transferred to a temperature of 60–65° F. after storage.

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602. EXPERIMENTS IN CHEESEMAKING WITHOUT STARTER

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(With 3 Figures)

For the preparation of good Cheddar, Cheshire and similar cheese a correct rate of acidification and degree of acidity is essential. Therefore the faults to which the starter organisms are prone may have severe consequences. For this reason it has often been suggested that alternative methods of acidification should be explored. A successful method would, at one stroke, cut the Gordian knot of bacteriophage, antibiotics, winter slowness and other difficulties.

According to Van Slyke & Publow (1) previous attempts to use dilute acid in place of starters had not been adequately controlled. Hucker & Marquardt (2) claimed to be able to produce normal cheese by using lactic acid in place of starter, but the experience of others as well as our own experiments show that the normal cheesemaking implied by Hucker & Marquardt cannot be achieved simply by the addition of acid. These workers were, moreover, equally successful in producing cheese without either starter or lactic acid, and this was doubtless due to their use of raw milk with the relatively high count of 500,000–1,000,000 bacteria/ml. It appears that their results were due to the initial flora of the milk acting as starter, and that therefore they do not indicate the successful use of artificial acidification.

More recently Mulder & Radema (3), making Edam cheese under difficulties of fuel shortage, added calcium chloride and hydrochloric acid to milk to improve its clotting properties. They continued the use of starter, but the added calcium and hydrogen ions permitted clotting and syneresis to take place at relatively low temperatures. The amount of hydrochloric acid they needed was less than one-tenth of that necessary to secure sufficient acidity for the proper ripening of English hard pressed cheese.

The experiments reported in this paper fall into two sections; the first concerns the preparation, without the use of a starter culture, of a curd with satisfactory physical properties, and the second concerns the improvement in flavour of the ripened product.

MATERIALS AND METHODS

Milk. A mixture of morning and evening milk was used in Exps. 1–4. Bulked morning milk was employed in the remaining experiments. In all cases the bacterial content was about 5000 organisms/ml. or less.

Pasteurization. Two methods were employed. The first (Exps. 19, 21, 22) involved passing warmed milk at about 500 ml./min. through two glass condensers, whose outer jackets were steam-heated, so that a maximum temperature of 68° was reached. After 1 or 2 sec. at this temperature the milk was cooled by passing it through coils immersed in cold water. Alternatively (Exp. 24) the milk was heated to 57° over 20 min., held at 57° for 3 min. and cooled.

Cheesemaking. The cheeses were made on a 14 gal. scale using a method based on the Cheddar and Cheshire processes. In most cases the curd was milled finely as for Cheshire cheese so that extra acid could easily be incorporated at this stage. Modifications were introduced as thought desirable. The cheeses were ripened at 55° F.

Estimation of quality of cheese. Cores were taken at intervals during the ripening period and tasted by a small group of workers (usually six) who were accustomed to assessing the flavour of cheese. Any cheeses which were thought to be satisfactory were then distributed to about twelve people who were not necessarily experienced in cheese-tasting.

EXPERIMENTAL

The techniques used and some of the observations made are recorded in Tables 1 and 2. The schedule of Table 3 is provided to form a concrete example of the process which was most frequently used in the later experiments, and to provide, together with Tables 1 and 2, a background for the notes which follow.

Preparation of a curd resembling that of Cheddar cheese. Normally the curd of Cheddar cheese before pressing has a pH of about 5·5, and this drops quickly to below 5 as the starter organisms continue to multiply and produce lactic acid. In the absence of starter organisms it was reasonable at first to try acidifying cheese-milk with lactic acid to control the growth of unwanted flora, e.g. coliforms.

In Exp. 1 commercial lactic acid (200 ml.) diluted with an equal volume of water was added during 2 min. with rapid agitation to milk at 86° F. to give a pH of about 5·4. A normal quantity of rennet was then added during 15 sec.; clotting took place after only 2 min. because of the low pH, and the curd was cut after 15 min. The usual Cheddar process was then followed. A silky, weak-bodied curd was obtained oozing whey under manual pressure. Under tension the curd particles were elastic and did not break cleanly like a normal curd. Little improvement in body took place on Cheddaring.

In an attempt to get a drier curd (Exp. 2) sufficient lactic acid to give a pH of only 5·8 in the milk was added before renneting; calcium chloride ($CaCl_2.6H_2O$, 200 g.) in aqueous solution was also added during scald to diminish the loss of calcium from the acidified curd in the hope that its physical properties would thereby be improved. Some improvement indeed took place. The Cheddared curd had a pH of 5·9, and it was necessary to consider how best to reduce this value so that it would reach about 5·0 after pressing. Gluconic acid lactone was chosen for this purpose; it hydrolyses to the free acid at a convenient rate (Fig. 1), it is readily available, non-toxic, and free from other objectionable properties such as insolubility of calcium salts and unpleasant taste. The lactone (165 g.) was added with the normal quantity of salt to the finely milled curd just before pressing.

Whether the improvement in curd properties in Exp. 2 resulted from the higher pH at renneting or from the addition of the calcium chloride was explored in Exp. 3. Here calcium chloride was again added, but the pH was adjusted to about 5·4 before the addition of rennet. The inferior curd obtained suggested that in the former experiment the higher pH at renneting was the more important factor and a similar pH was consequently employed in succeeding work.

In Exp. 4 hydrochloric acid was used instead of lactic acid for reasons of economy. Its use did not apparently affect the physical properties of the curd. The higher pH at

Table 1

Exp. no.	Additions to milk: Lactic acid (ml.)	Hydrochloric acid (ml. 3·3 N)	Gluconic acid lactone (g.)	Calcium chloride (g.)	Scald: Rennet (pH)	Calcium chloride (g.)	Hydrochloric acid (ml. 2 N)	Time (min.)	Final temp. (° F.)	Pitching: pH whey	pH curd	Addition to curd: Gluconic acid lactone (g.)	Salt (oz.)	Comments about curd
1	200	—	—	—	5·35	—	—	—	—	5·6	5·30	—	7	Silky. Weak body. Rubbery
2	133	—	—	—	5·82	200	—	35	100	—	5·90	165	4	Firmer than Exp. 1. Rubbery
3	180	—	—	—	5·34	100	—	—	—	—	5·30	—	—	Lacking body. Curd membrane elastic
4	—	180	—	200	5·76	—	90	—	—	5·48	—	165	5	Similar to Exp. 2
5	—	240	—	—	5·85	200	—	—	—	—	—	165	4	Good body
6	—	240	—	—	5·80	—	—	—	—	—	—	165	5	Similar to Exp. 5
7	—	240	—	—	5·95	—	105	40	—	5·57	5·82	165	5	Too shotty
8	—	270	—	—	5·80	—	120	32	98	5·46	5·80	165	5	Very shotty
9	—	255	—	—	5·88	—	120	28	94	5·73	—	165	5	Too wet
10	—	255	—	—	5·96	—	120	45	94	5·70	—	165	5	Shotty. Wet inside particles
11	—	255	—	—	—	—	120	63	86	—	—	165	5	Shotty and would not mat
12	—	240	—	—	6·02	—	105	43	98	5·78	6·01	165	5	Shotty, but matted
13	—	240	—	—	6·02	—	105	70	98	5·74	6·00	165	5	Slightly shotty, matted well, tore well
14	—	66	—	—	6·52	—	150	40	98	6·0	—	215	5	Shotty, inferior to normal curd
15	—	60	160	—	6·47	—	105	60	98	5·68	5·90	165	5	Normal at cutting. Tough at milling
16	—	60	160	—	6·46	—	—	68	98	5·92	6·06	165	5	Pitched normally. Slightly brittle
17	—	60	360	—	6·60	—	—	40	98	—	5·84	165	5	Silky. Good hot-iron test

Table 2

Exp. no.	Additions to cheese milk: Hydrochloric acid (ml. 3·3 N)	Gluconic acid lactone (g.)	Trypsin (g.)	Acetic acid (ml. 5 N)	*L. casei* 2d. 30° (ml.)	*L. brevis* 2d. 30° (ml.)	Scald: Hydrochloric acid (ml. 2 N)	Time (min.)	Final temp. (° F.)	Whey drawing: Whey	Curd	Gluconic acid lactone (g.)	Salt (oz.)	Comments about curd
18	60	160	—	—	120	—	105	50	98	5·6	6·02	165	5	Normal
19	60	350	—	—	70	—	—	46	98	—	—	165	5	Hot-iron test good. Rough tear
20	60	350	1·0	—	—	—	—	54	98	—	—	165	5	Hot-iron test very good. Rough tear
21	—	350	1·0	60	120	—	—	45	98	—	—	165	5	Very buttery odour at milling
22	60	360	1 l. tryptic digest	—	20	—	—	43	98	5·55	—	165	5	Very good at cutting stage. Handled well at pitching
23	60	360	—	—	40	40	—	46	98	5·55	—	165	5	Some floated at pitching
24	60	360	—	—	5	5	—	45	98	—	5·78	165	5	Slight floating at pitching

rennetting had, however, introduced the difficulty of obtaining a sufficiently low pH at the milling stage. To overcome this, hydrochloric acid (2 N, 150 ml.) was added slowly during the scalding. The curd behaved normally until near the end of the scald when it suddenly became 'shotty', presumably because the main action of acid had been on the outer surfaces of the curd particles. Subsequent work (Exp. 5–13) consisted mainly in various modifications of the process of Exp. 4 (see Table 1). No modification gave a pitched curd with normal properties.

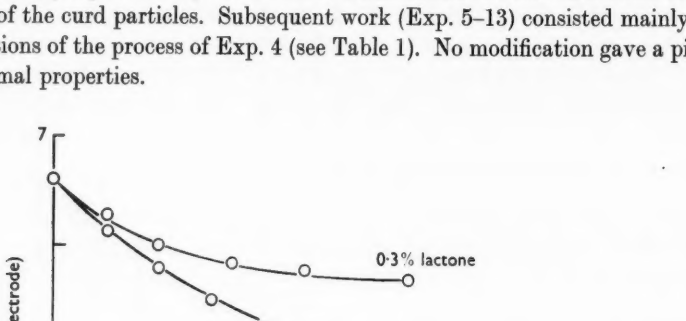

Fig. 1. Rate of hydrolysis of gluconolactone in milk.

Table 3. *Schedule of operations in making cheese without starter*

Time after beginning hr.	Time after beginning min.	Approx. period of time for operation	Operation
0	0	12 min.	4·3 ml. 12% HCl per gal. milk added slowly with vigorous agitation
0	12	20 sec.	While agitation continues 25·7 g. gluconic lactone powder per gal. milk sprinkled on to the surface of the milk
		30 sec.	Normal quantity rennet added and stirred in
0	13	30 min.	Curd allowed to set
0	43	5 min.	Cut in normal manner (as for Cheddar or Cheshire)
0	48	20 min.	Curd is stirred
1	8	20 min.	Temperature raised at 0·3° F. per min. to 92° F.
1	28	12 min.	Temperature raised at 0·5° F. per min. to 98° F.
1	40	10 min.	Curd allowed to settle
1	50	5 min.	Whey drawn off
1	55	75 min.	Curd cut, piled and drained in usual way
3	10	—	Milled and pressed as usual but with addition of a mixture of gluconic lactone and salt in place of the usual salt alone. Amount of lactone: 11·4 g. to the curd from each gallon of milk

It had initially seemed probable that the rate of acidification during the 'make' would have an important effect on the physical properties of the curd. The experience obtained from Exps. 1–13 supported this hypothesis. Accordingly an attempt was made to adjust the pH with hydrochloric acid at all stages to values similar to those obtaining in the

Cheddar process (Exp. 14). A control cheese was made by the normal process to allow comparative observations to be made throughout. It was noted that the curds differed from each other from the very beginning of setting. Pools of whey appeared on the surface of the 'non-starter' curd, and it was soft, weak in body and readily parted from the sides of the vat. The conclusion was reached that not only was the rate of acidification important but that the acidification must take place within the curd particle. It was here that gluconic acid lactone would be most useful.

The rate of hydrolysis of gluconic acid lactone in milk had already been determined (Fig. 1). From this it was calculated that a reasonable approach to normal conditions of acidity in the curd would be made by an initial acidification of the cheese-milk with hydrochloric acid to a pH of about 6·4 followed just before renneting by enough gluconic acid lactone to give a drop of 0·4 pH unit in the curd during the setting period. In Exp. 15 an acidification within the curd particles was attained by such a process. The resulting curd was normal; it had a good body, did not shrink from the sides of the vat and had no whey on the surface. The difficulty of acidifying the curd during the scalding still remained. In this experiment 2 N-HCl (170 ml.) was added at about 2·5 ml./min. during the scalding period; although this was effective in reducing the pH of the whey the pH of the curd particles remained high.

As the value of acidification during the scalding was doubtful it was omitted in Exp. 16. The salted curd was, however, treated as usual with gluconic acid lactone before pressing. When a sample taken just before pressing was held at room temperature overnight its pH fell to 5·18. This is to be compared with the much slower rate of acidification if lactone is omitted at the salting stage; samples of curd which were not treated with lactone remained at about pH 5·8 for 48 hr. After this time the natural flora from the raw milk brought about a significant fall in pH. It would, therefore, appear that any lack of control over the microflora could be attributed partly to the relatively high pH's existing in the curd between cutting and pressing.

In Exp. 17 the effects on the curd of incorporating various amounts of hydrochloric acid and lactone in the cheese-milk were measured by bowl readings (4). Increasing the lactone to 360 g. still allowed the time of cutting to be selected without difficulty at a normal bowl reading of about 65 (Fig. 2) and had the advantage of producing a lower pH in the curd at pitching. Consequently most of the subsequent experiments employed 360 g. lactone and 20 ml. HCl in 14 gal. milk.

Quality of the ripened cheese. The textures and flavours of the finished cheeses are described in Table 4. Table 5 shows the results obtained when a small group of independent observers were asked to taste selected products. Since most of the cheeses were made according to Cheshire practice, the crumbly texture is normal.

Development of flavour in non-starter cheese. It was soon apparent that in those cheeses which were acceptable the flavour was, without exception, very mild and it did not persist in the mouth like the normal flavour. Various methods of improving the flavour were therefore tried. They consisted mainly in an attempt to encourage the development of lactobacilli in the cheese.

Initially (Exp. 18) raw cheese-milk was inoculated with a strain of *Lactobacillus casei* whose fate could be followed serologically during the cheese ripening (5). Subsequently (Exp. 19) pasteurized milk was used and the size of the inoculum reduced. Various additions were also made to the cheese-milk with a view to improving the curd as a growth

medium for lactobacilli (Exps. 20–22). Later *L. brevis* was also included in the inoculum (Exps. 23–24).

The maximum number of *L. casei* which was found in the ripening cheese was about 10^8/g. and appeared to be independent of the size of the inoculum; the maximum was

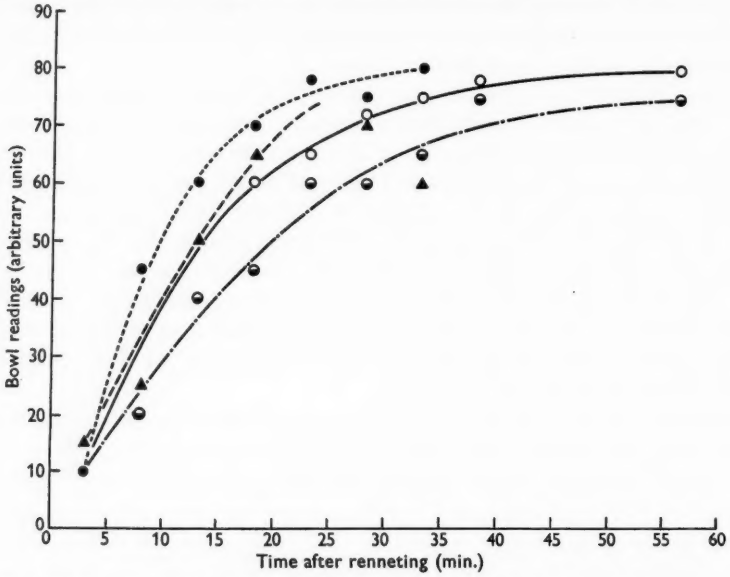

Fig. 2. Effect of different amounts of gluconolactone and/or hydrochloric acid on the consistency of cheese curd as measured by the bowl method. In 14 gal. milk:—◒ 25 ml. 1 0N HCl; ○, 17·5 ml. HCl, 165 g. lactone ● 20 ml. HCl, 360 g. lactone; ▲, 360 g. lactone.

Table 4. *Flavour and texture of cheese made without starter*

Exp. no.	Age of cheese (months)	Texture	Flavour
1	12	Good	Slightly acid, slightly cheesy
2	6	Crumbly	Beery, slightly cheesy
3	5	Blown	Slightly acid, fruity, beery
4	5	Dry, crumbly	Very mild, pleasant, transitory
5	3	Blown, soft, crumbly	Beery
6	3	Blown, soft, crumbly	Beery
7	5	Crumbly	Slightly acid, pleasant
8	3	—	Acid, pleasant
9	5	Crumbly	Slightly acid
10	8	Very good	Slightly cheesy, like 7
11	8	Crumbly	Very acid, fruity
12	7	Crumbly	Rancid, slightly cheesy
13	5	—	Acid, bitter
14	8	—	Potato aroma, slight putrefaction
15	6	Slightly crumbly, satisfactory	Mild, slightly bitter, like processed cheese
16	6	Some gas holes	Very acid, fermented
17	5	Good	Nutty, slightly cheesy
18	6	Hard	Acid, fruity
19	5	—	Very acid, slightly bready, unpleasant
20	4½	Slight blowing	Vinegary, fruity
21	4	Hard	Acid, very fruity
22	6	—	Acid, fruity, pleasant
23	6	—	Acid, fruity, pleasant
24	4	Crumbly	Acid, pleasant

Table 5. *Summary of results of tasting of non-starter cheese by a group of disinterested observers*

	Do you like it?				Do you prefer it to?–			
	Raw		Cooked		Ration cheese		Processed cheese	
Cheese no.	+	–	+	–	+	–	+	–
4	9	3	4	4	7	4	7	5
7	7(2)	1	5	1	5(2)	3	7(1)	2
9	7(2)	4	5(2)	2	4	10	9	5
10	7(1)	5	5(2)	4	2	8	5	4
15	3(2)	3	2(2)	2	1	7	6	2

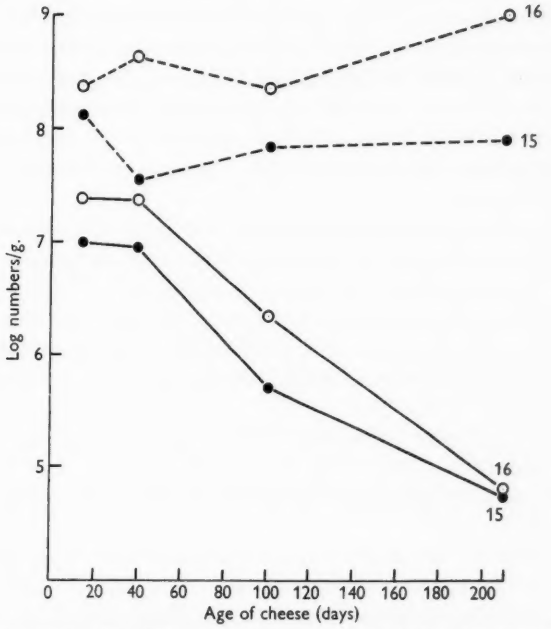

Fig. 3. Death rate of staphylococci in cheeses No. 15 and 16 made without starter.
---- Total numbers of organisms; —— Numbers of staphylococci.

more quickly attained when the inoculum was high. *L. brevis*, although growing at the same rate as *L. casei* in the first 2 weeks failed to reach such high numbers.

When the samples taken for bacteriological analysis were tasted, a mature Cheddar flavour was occasionally noted in the early ripening period, e.g. Exps. 18 (3 weeks), 19 (2 weeks), 21 (4 weeks), 34 (6 weeks). The flavour appeared to be transient and could not be found in later samples from the same cheese. No permanent improvement in flavour appeared to be gained, therefore, by inoculating with these strains of lactobacilli.

Development of undesirable organisms in non-starter cheese. The omission of starter inoculum and the relatively high pH of the curd between cutting and milling removes much of the normal control over organisms such as coliforms and staphylococci. For instance, in cheeses 15 and 16, which had been made with raw milk, staphylococci were found in large numbers after 2 weeks of ripening, but they gradually died out (Fig. 3).

The trouble may have been associated with the high numbers of staphylococci in the milk being used at that time. These organisms have not been found in large numbers in non-starter cheese made from pasteurized milk.

Little trouble has been experienced from coliform organisms. Cheeses 18–24 were examined periodically. In the case of the raw-milk cheese 18 the coliforms decreased from 100,000 $g.^{-1}$ at 3 days to 1000 $g.^{-1}$ at 6 weeks, and in the pasteurized milk cheese they exceeded 100 $g.^{-1}$ only on one occasion.

DISCUSSION

A continuous, slowly developing acidity within the curd during the setting stage of Cheddar cheesemaking appears to be essential for good curd texture. Thus the use of gluconic acid lactone, which hydrolyses slowly to produce this acidity, overcame the initial problem of producing a suitable curd without the use of a starter inoculum.

It is improbable, however, that an important stage in a biological process can be replaced by a non-biological system without modifying the resultant products. The substitution of an acid lactone for the starter organisms in cheesemaking modifies the process in two main ways:

(*a*) The hydrolysis of the lactone and hence the production of acid takes place at a maximum rate immediately the lactone is added, i.e. at renneting, and decreases asymptotically. During scalding therefore little acid is being produced within the curd particles when starter is absent; this constitutes a major difference between the gluconic lactone and starter processes, because in the latter the starter organisms reach a peak acid production which is maintained during the setting of the curd and for some time after cutting.

(*b*) The absence of starter activity in cheese made with lactone results in a curd containing a higher lactose content than normal. This would be expected to influence the microflora developing during the ripening period. The effect of washing the curd has not yet been explored.

No simple method for the satisfactory acidification of the curd during the scalding process has yet been found. Consequently the growth of coliforms and other undesirable organisms might occur in this period in the absence of any control due to acidity or to the competition from starter bacteria. A detailed study of the flora of cheese made with gluconic acid lactone still remains to be done. In a few of the cheeses which were examined, multiplication of staphylococci was noted, but the resultant cheeses were safe to eat. No development of undesirable flora has been noted in cheese made from pasteurized milk.

The cheeses made without starter had generally two properties in common; the texture was crumbly and the flavour acid. No Cheddar flavour developed in any of the cheese, but it was very noticeable that the flavour improved after cooking or toasting. Inoculations with *L. casei* or a mixture of *L. casei* plus *L. brevis* failed to produce a Cheddar flavour, although the organisms grew satisfactorily. It could be inferred that the products of the starter are important in the production of good flavour, but it has also to be remembered that the lactobacilli inoculated into these cheeses were probably utilizing lactose as a carbon source. The carbon source of lactobacilli in normal Cheddar cheese is not known, but no lactose is present at the time when they are developing vigorously. Thus

the products of their fermentation in normal cheese may be quite different from those formed in the 'non-starter' cheese.

The experiments which have been described were carried out mainly to discover whether methods of artificial acidification were worth exploring. For this reason no elaborate measurements of the properties of the curd or of the cheese were undertaken; an attempt was made to produce curd which appeared normal to the observer. In this success was achieved and it is clear that the way lies open for the prosecution of further research with the aid of more refined techniques. It is hoped that the absence of starter organisms will permit a closer study both of the behaviour of curd in the vat and of the effect of various added micro-organisms and enzymes on the ripening of cheese.

Further experiments are necessary before it can be decided whether the obvious advantages of speed and regularity are worth the cost of the materials; moreover it is not yet known whether the flavour of such cheeses would be generally acceptable. On the other hand, it is quite likely that occasional starter failure could be successfully countered by the use of gluconic lactone.

SUMMARY

Cheese has been successfully made from raw and pasteurized milk by a process similar to that employed for Cheddar or Cheshire-type cheese but omitting the starter inoculum.

In the preferred method gluconic acid lactone was used as the acidifying agent during the setting of the curd and after milling.

The flavour of the best products was acceptable to a number of people not necessarily experienced in cheese-tasting, but it was different from that of normal Cheddar.

No improvement in flavour was achieved by inoculation of the cheese milk with a strain of *L. casei* and *L. brevis*.

Further detailed investigation is needed to perfect the process and to determine whether its use in industry is justified or desirable.

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637·333·135

603. DEVELOPMENT OF SEROLOGICALLY IDENTIFIED LACTOBACILLI ADDED TO CHEESE MADE WITHOUT STARTER

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Lactobacilli are found in large numbers in cheeses of many varieties, and it is therefore believed that they are concerned with the development of flavour. In investigating this, Mabbitt (1) made cheese without starter, adding strains of lactobacilli to the cheese milk to determine whether flavour would then develop. The serological types of the lactobacilli were known, and the present paper describes the serological methods of identifying the strains of lactobacilli which were recovered from the cheese with those which were added.

MATERIALS AND METHODS

Organisms. Strains of lactobacilli used were *Lactobacillus casei* N.I.R.D. H831 (C6) and *L. brevis* N.C.T.C. 6107 (X1). Both were old laboratory strains maintained in chalk yeast dextrose litmus milk.

Cultures added to cheese milks. Cultures were grown for 48 hr. in 500 ml. tomato juice yeastrel Tween 80 broth (T.D.B.) described by Briggs (2). The washed centrifuged cells were then inoculated (Mabbitt (1)) into appropriate cheese milks. Cultures of C6 were added to cheeses 18A, 19, 21 and 22, and a mixture of C6 and X1 in approximately equal numbers of organisms, to cheeses 23 and 24. The inoculum of lactobacilli cells in cheeses 18, 19 and 21 was approximately 100 × that in cheeses 22 and 23 and 400 × that in cheese 24.

Isolation of lactobacilli from cheese. 9 g. of cheese were homogenized in 80 ml. citrate, serial dilutions made, plated in duplicate in tomato juice yeastrel Tween 80 agar (Briggs (2)), and incubated anaerobically for 3 days and counts made. Ten per cent of the colonies, or ten colonies, which ever was the greater, were picked at random into 10 ml. T.D.B. from each of the duplicate plates of a suitable dilution, making in all at least twenty colonies from each sample. When growth had occurred, cultures were tested for catalase production, and all catalase-negative organisms subcultured into 10 ml. T.D.B. without yeastrel, for HCl extraction. In the first experiments the catalase-negative cultures were examined microscopically, but as only lactobacilli were observed this was discontinued.

The first samples examined from cheeses 18, 19 and 21 were taken 5–10 days after making the cheeses; the first samples examined from cheeses 22, 23 and 24 were the cheese curds before pressing. After that, samples were taken at irregular intervals.

The incubation temperature for all cultural work was 30° C.

Serological methods

Preparation of type sera. Antisera were prepared according to the method described by Sharpe (3) for the preparation of group sera for lactobacilli, except that in the case of C 6, suspensions of cells for inoculation were heated to 65° C. for 1½ hr. to destroy the

group antigen. A course of five twice-weekly inoculations produced potent specific type sera in each case.

HCl extracts. Lancefield's (4) method was used. Cultures were grown for 24 hr. in 10 ml. T.D.B. without Yeastrel (Sharpe (3)), and the cells extracted with 1·0 ml. of 0·05 N-HCl in saline in a boiling water-bath and neutralized to phenol red.

Precipitin tests. Ring precipitin tests were used.

RESULTS

Organisms isolated at the first dilution which was countable. In addition to lactobacilli a few micrococci were isolated from the first sample of cheese 18, and a few Gram-negative bacilli from that of cheese 21. All subsequent samples of these two cheeses contained only lactobacilli at the dilutions examined. From the other four cheeses only lactobacilli were isolated.

Identification of lactobacilli. As extracts of all lactobacilli isolated gave strong precipitin reactions with the specific type sera, it was possible to identify them all. Lactobacilli isolated from cheeses 18, 19, 21 and 22 were typed as C6; lactobacilli isolated from cheeses 34–37 were identified either as C6 or X1, the proportional numbers of these varying with the age of the cheeses.

Numbers of lactobacilli present. Although the number of lactobacilli cells inoculated into the cheese milks varied with each cheese, the maximum number of organisms enumerated was in each case about 10^9/g., independent of the size of the inoculum (Table 1). After 7–8 weeks the numbers declined steadily, but even after 35 weeks (cheeses 18 and 19) large numbers were still present.

Table 1. *Total numbers of serologically identified lactobacilli in cheeses made without starter*

Age of cheese in weeks	Counts × 10^7 of lactobacilli/g. in cheeses					
	18	19	21	22	23	24
0	—	—	—	0·2	0·51	0·11
1– 2	6	42	168	125	17	72
3– 4	120	191	230	—	—	—
6– 7	130	—	—	195	149	—
8–12	26	108	221	—	78	—
13–18	10	37	65	110	—	147
25–30	—	—	23	12	6	60
30–36	0·8	4	—	—	—	—

Table 2. *Numbers of strains of* Lactobacillus casei *and* L. brevis *serologically identified in cheeses made without starter*

Age of cheese in weeks	Counts × 10^7 of lactobacilli/g. in cheeses			
	23		24	
	L. casei	*L. brevis*	*L. casei*	*L. brevis*
0	0·19	0·32	0·04	0·07
1–2	14	3	62	9·8
7	144	5	—	—
10	74	4	—	—
18	—	—	147	>1
23–25	5·4	0·3	59	>1

Table 2 shows the proportion of C6 to X1 in the two cheeses in which they were both present. Although in each case there were initially slightly more X1 organisms present

than C6, after 2 weeks the numbers of C6 exceeded those of X1 and continued to do so throughout the sampling period. There was no indication that the homofermentative C6 organisms multiplied in the first stages and the heterofermentative X1 in the later stages of cheese ripening. Both organisms multiplied in the first stages, but C6 more rapidly than X1.

DISCUSSION

This method of following the development of organisms in cheese by serological techniques, although used only on a small scale here and in cheese made without starter, offers interesting possibilities for future work on the flora of cheese. The starters themselves could be of known serological types if it were desired to follow their progress. Other organisms could be similarly identified and their development studied in cheese. The use of pasteurized milk and sterilized equipment would largely obviate extraneous organisms.

The two strains of lactobacilli studied did not give rise to a typical Cheddar flavour in the cheeses (Mabbitt (1)). It is not known whether the use of unsuitable strains or unfavourable conditions in the cheeses may have contributed to this lack of flavour production.

I wish to thank Dr A. T. R. Mattick for initiating this work; Dr L. A. Mabbitt for his helpful co-operation and Miss P. Burrows for technical assistance.

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604. THE IMPORTANCE OF LACTOBACILLI IN THE PRODUCTION OF FLAVOUR IN CHEDDAR CHEESE

I. GROWTH OF LACTOBACILLI IN CHEESE SERUM

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(With 2 Figures)

Despite many investigations over a long period (reviewed recently by Pette(1), Kosikowsky(2)), no positive evidence has been revealed concerning the identity of the substances responsible for the typical flavour of Cheddar cheese. Nor is it known for certain whether or not the typical flavour is a product of bacterial metabolism. Since this flavour becomes marked only after several months of ripening, it is natural that much attention should be paid to the population of lactobacilli which generally become dominant at about this time (Davis(3)).

If the hypothesis that the metabolism of lactobacilli in cheese results in the production of flavouring compounds is provisionally accepted, it becomes of considerable interest to ascertain the metabolic products of these organisms in cheese.

Lactobacilli are known to prefer the simpler products of protein breakdown (Davis & Mattick(4)), and since their development in Cheddar cheese takes place at a time when such substances are freely available, it is reasonable to suppose their nitrogen requirement is met from this source. We have, however, no knowledge as to the carbon source of lactobacilli in Cheddar cheese. At the time that they normally multiply significantly, all free sugar has been exhausted (van Slyke & Bosworth(5); Fagen, Stine & Hussong(6)) by the starter organisms. Lactate is, however, present in high concentration at this time, and it has been suggested as a possible carbon source (Orla-Jensen(7)). No lactobacillus isolated from normal cheese has, however, been found capable of fermenting lactate *in vitro*(3).

This paper reports on preliminary experiments designed to throw light on this problem. It was assumed that the carbon source in cheese was present as solute in the aqueous phase. Observations were, therefore, made on the growth of lactobacilli in expressed aqueous cheese juice (serum).

MATERIALS AND METHODS

Serum. This was prepared by expressing the juices from a mixture of cheese and sand as described by Mabbitt(8), and unless otherwise stated the cheeses used here were the same.

Test organism. Generally a strain of *Lactobacillus brevis* isolated in 1953 from Cheddar cheese, and maintained in glucose-yeastrel agar stab culture, was used; it is referred to as T.C. 9.

Other lactic acid bacteria used were either from dried cultures in the Institute's collection or were fresh isolates from the cheese under investigation.

Growth tests with the serum. An 18 h. culture of the organism to be tested was centrifuged, washed with saline and resuspended in saline. An aliquot of the suspension was plated on glucose-yeastrel agar and a second aliquot used to inoculate 0·5 or 1·0 ml. quantities of serum contained in small tubes; the inoculum was usually about 200 cells ml.$^{-1}$ serum.

The cultures were normally incubated in sealed jars filled with a mixture of nitrogen containing 5% (v/v) of carbon dioxide for 5 days at 30°, and the extent of growth estimated by plating as before.

Salt content of the serum. The method outlined by the British Standards Institute Bulletin (9) was used.

Osmotic pressure of the serum. This was estimated by measurement of the freezing-point of the serum, using a Beckmann thermometer.

Bacterial counts. Cheese (10 g.) taken aseptically with a sterile borer was homogenized with 2% w/v sodium citrate solution (90 ml.) and dilutions plated on glucose-yeastrel agar. A total count was made and an estimate of the number of lactobacilli present was obtained by a random selection of colonies.

RESULTS

Growth of lactobacilli and other organisms in serum from 15*-month-old cheese* (C_{15})

The cheese was of good flavour. T.C.9 not only failed to grow in the serum but the medium was bactericidal. The organisms survived for about 7 hr. but died rapidly thereafter. The growth of other organisms in the serum was also tested; they included *L. casei* (6 strains), *L. lactis* (8 strains), *L. acidophilus* (1 strain), *L. plantarum* (4 strains), *Streptococcus faecalis*, *Leuconostoc* sp. and a micrococcus isolated from cheese. The serum was in all cases either bactericidal or bacteriostatic.

General occurrence of the inhibitory phenomenon

Three manufactured cheese probably about 6 months old, selected at random, and two others from the Institute's dairy provided serum having similar inhibitory properties to that from C_{15}. Further investigation of the phenomenon was therefore undertaken using serum from C_{15}.

Nature of the inhibitory substance(s)

Preliminary observations

It was possible that the acidic cheese juices were causing corrosion of the press cylinder and that dissolved metal was responsible for the bactericidal activity of the serum. Impurities in the sand used for pressing might also be involved. Neither of these explanations was found to be tenable. For example, the juice could be prepared by an alternative process involving homogenization of the cheese with water, centrifuging to obtain the clear aqueous layer and concentrating under vacuum to remove added water; the product had bactericidal properties similar to those of the serum.

The effect on growth of various additions to or treatments of the serum are shown in Table 1. Although the addition of a carbon or nitrogen source failed to overcome the inhibition, the presence of both sources together allowed limited multiplication of the organism (about 20×). A much more pronounced improvement in growth was observed after autoclaving the serum. The heat treatment was accompanied by a fall in pH from

Table 1. *Effect of various additions to and treatments of cheese serum on its suitability for growth of* Lactobacillus brevis

Treatment	Addition	Nos. of *L. brevis*	
		Before incubation	After incubation
—	6% glucose	200	0
—	6% peptone	200	0
—	Casein digest	200	0
—	6% glucose + 6% peptone	200	4,400
—	6% glucose + casein digest	200	3,180
—	0·008% catalase	1,728	0
Autoclave 20 min. at 121°	—	1,728	>20,000

5·42 to 5·25, so that changes in acidity could not explain the improvement. Similar effects of heat treatment on staled medium have been previously reported (Eijkman (10)), and it seemed probable that a similar phenomenon was being observed here. Subsequent experiments, however, showed either a very feeble or no effect on autoclaving and investigation along this line was discontinued. The addition of catalase to the serum did not destroy its bactericidal properties and it was concluded that peroxide was not the inhibitory factor.

Attempts to demonstrate the inhibitory activity of the serum by the cup and plate technique using glucose-yeastrel agar seeded with T.C. 9 were not successful. No zones of inhibition could be demonstrated, even when time was allowed for diffusion of the serum into the agar by overnight storage at 4° before incubating at 30°. Nor could inhibition be demonstrated by filling a trough in a glucose-yeastrel agar plate with a mixture of 1 part serum + 1 part double-strength agar and cross-streaking the test organism.

Concentration of the inhibitory substances in the serum

Initial experiments showed that a dilution of the serum with an equal volume of sterile water allowed growth of T.C. 9. A more detailed study gave the results which are summarized in Fig. 1. The addition of increasing quantities of water to the serum gradually improved its growth properties until after being diluted two-fold it permitted the growth of about 10^7 organisms ml.$^{-1}$. It was at first thought that the flat portion of the curve AB indicated that two compounds or groups of compounds were involved, but repeated experiments failed to confirm this observation, although the general shape of the curve was reproducible.

Available carbon and nitrogen sources appeared to be present in the serum in adequate concentrations. Thus, satisfactory growth could be obtained even after an eighty-fold dilution with water.

Salt content and inhibition

The salt content of the serum was found to be 4·8% (w/v), and it seemed possible that this might account in some measure for the growth-inhibiting properties of the serum. The survival or death of those organisms previously tested in the serum bore, however, no relationship to their salt tolerance. Also when the serum was diluted with an equal volume of 4·8% saline, it supported growth of T.C. 9.

It was concluded that other factors beside the salt content were involved in the inhibitory phenomenon and that further information was required before studies of the metabolism of lactobacilli in the serum could be initiated.

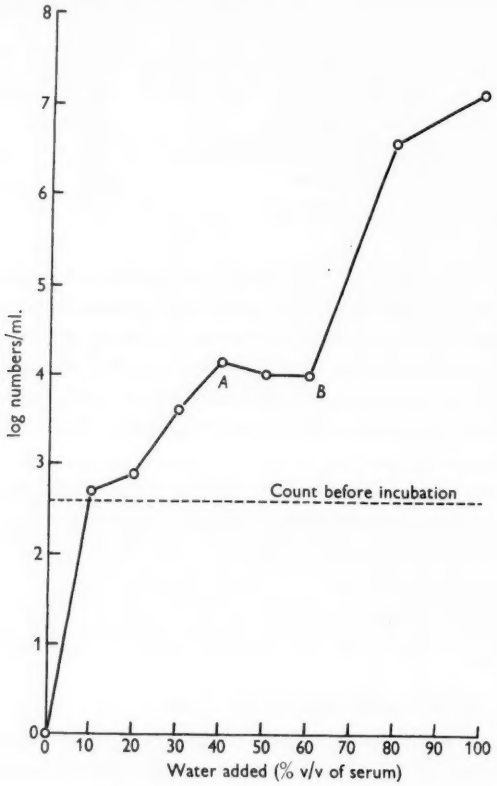

Fig. 1. Growth of *L. brevis* T.C. 9 in Cheddar cheese serum diluted with various quantities of water.

The importance of fat globules

The Seitz-filtered serum, although transparent, contained tiny fat globules which could be seen easily under the oil-immersion lens of a microscope. To examine the possibility that they were in some way responsible for the inhibitory phenomena, a sample of expressed fat from cheese C15 was shaken with yeastrel-glucose broth, centrifuged and passed through a Seitz filter. Microscopic examination of the filtered liquid showed that it contained fat globules similar in number to the inhibitory serum, but it was found to support good growth of T.C. 9.

It was concluded that the inhibitory factor in cheese was not associated with the fat fraction.

Possible contribution of amino-acids to the inhibitory effect

When alcohol was added to the serum to give a final concentration of 75% (v/v) the precipitate, consisting mainly of peptides and proteins, when added to glucose-yeastrel broth conferred no inhibitory properties on the medium. On the other hand, the alcoholic

extract when dried under vacuum and added to broth caused inhibition of T.C. 9. The inhibitory factor was, therefore, soluble in 75% alcohol. This suggested that it would be worth while to examine the possibility that amino-acids were concerned in the inhibition.

The inhibitory effect on microbial growth of an imbalance or excess of amino-acids in the medium has been previously reported for *Bacillus anthracis* (Gladstone (11)) and some lactobacilli (Brikson, Henderson, Sohljell & Elvehem (12); Teeri & Josselyn (13)). To examine the possibility that a similar phenomenon was responsible for the inhibitory properties of the cheese serum, samples were taken at intervals from cheese S1, whose amino-acid content was being determined (8), and the growth properties of the serum ascertained with respect to *L. brevis* T.C. 9 and to a lactobacillus strain freshly isolated from the cheese (Table 2). Total counts and counts of lactobacilli were also made on the same sample of cheese (Fig. 2).

Table 2. *Multiplication of lactobacilli in the Seitz-filtered serum of Cheddar cheese S*1 (4 *days at* 30°)

	Final count/Initial count			
	L. brevis T.C. 9		Freshly isolated lactobacilli	
Age of cheese (months)	Undiluted serum	Diluted serum	Undiluted serum	Diluted serum
0·7	1,000	—	—	—
2·0	0·4	150	—	—
3·0	40·0	—	10,000	—
3·5	2·6	4,000	0·5	3,000
4·0	40·0	10,000	28·0	90,000
5·0	2,500·0	3,800	—	—
6·0	2·0	10,000	—	—
7·0	0·5	200	—	—

It is seen that the microflora initially consisted mainly of homofermentative and heterofermentative streptococci whose numbers closely followed the total count. At about 4–5 months this count reached a minimum and thereafter any increase was mainly due to growth of lactobacilli.

The growth-supporting properties of the serum towards *L. brevis* T.C. 9 resembled those towards the few freshly isolated strains which were tested; it became minimal at 3–4 and 6–7 months, and reached a maximum at 5 months. The shape of the curve showing the multiplication of T.C. 9 in the serum was similar to that illustrating the growth of lactobacilli in the cheese (Fig. 2), but the significance of this observation is not clear.

A continuous increase in osmotic pressure of the serum throughout the cheese ripening would be expected as a result of proteolysis and carbohydrate fermentation. If this were solely responsible for the inhibitory phenomena, it is not possible to explain the occurrence of the first period of inhibition followed by a period of normal growth.

Between the inhibitory period at 3½ months and the period of good growth at 5 months, however, the variation in the percentage increments of the different amino-acids was considerable (8). In particular, leucine, isoleucine and valine, whose imbalance is already known to affect adversely the growth of lactobacilli (12), increased by 175, 228 and 160% respectively. During the same period the glycine content increased by 20% and the glutamic acid by 220%. It is therefore not improbable that the ratios of the amino-acids were unfavourable to growth at 3½ months and favourable to growth at 5 months.

After a ripening period of 6 months the osmotic pressure of the serum from S1 cheese was equivalent to 9·45% sodium chloride. Its salt content was, however, only 4·8%, so that other constituents of the serum must have produced an osmotic pressure equivalent to 4·65% salt. On the basis of their concentration, the amino-acids in the serum (8) would be expected to contribute a pressure equivalent to at least 3·8% and were consequently, apart from the salt in the cheese, the major contributors towards the total osmotic pressure.

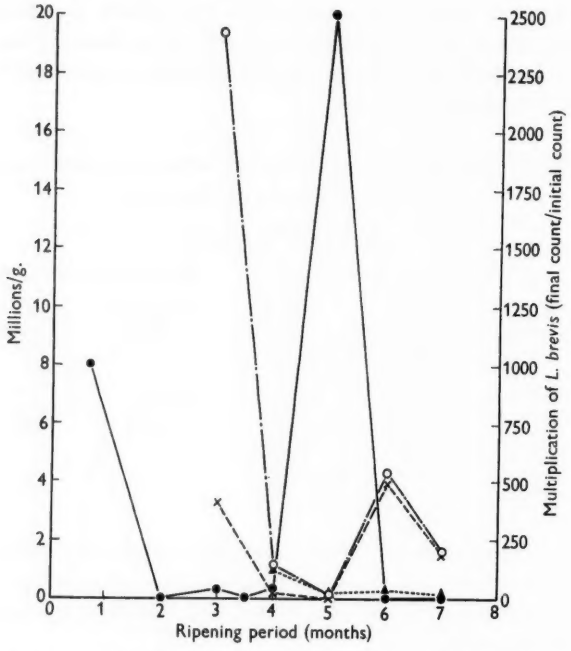

Fig. 2. Numbers of micro-organisms in Cheddar cheese (S1). ○—·— Total count; × – – – Lactobacilli; ▲- - - - Streptococci; and the multiplication of *L. brevis* T.C. 9 in the Seitz-filtered cheese serum ●——.

The inhibitory properties of the serum which appeared at 6 months could well be due to this increasing osmotic pressure. T.C. 9 was found not to grow in yeastrel-glucose broth containing 7·5% sodium chloride and having a total osmotic pressure equivalent to 9·5%. It has also been previously shown that *L. brevis* from cheese can grow in tomato-glucose broth containing 6% sodium chloride (i.e. total osmotic pressure equivalent to about 9%), but not if 8% NaCl is present (Briggs (14)).

Other mechanisms resulting from the high amino-acid content of the serum may also have been important. For example, an excess of several amino-acids, including glutamic acid, equivalent to 2 mg. ml.$^{-1}$ medium, is known to prevent growth of *L. casei* (13); the increase in glutamic acid in the 5–6-month period amounted to 4 mg. ml.$^{-1}$ serum.

The hypothesis that amino-acids were in some way responsible for the inhibitory properties of the serum is in accord with the previous observation that the inhibitory factor was soluble in 75% alcohol.

SUMMARY

1. The serum prepared by subjecting Cheddar cheese to pressure becomes inhibitory to the growth of lactobacilli as the cheese ripens.

2. One factor responsible for the inhibitory phenomena is the increasing osmotic pressure of the serum due mainly to the increasing amounts of amino-acids present.

3. Observations made during the ripening of a normal Cheddar cheese suggest that other factors, e.g. amino-acid imbalance, are also important.

We wish to thank Miss Helen R. Chapman for making the cheese and Dr R. Aschaffenburg for making the freezing-point determinations.

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637.333.33:577.156.5

605. PREPARATION OF A CHEESE WITH CRYSTALLINE RENNIN

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Kizer, Hankin, Speck & Aurand (1) have observed that the impurities associated with proteolytic enzymes are stimulating to lactic acid bacteria, and they have suggested that these substances may accelerate the ripening of dairy products. In view of these observations and suggestions an experiment begun in March 1954, on the preparation of cheese with crystalline rennin, is reported below. The results are in agreement with the suggestions of Kizer *et al.*

Three 30 lb. Cheddar cheese were made from a single batch of milk. One control cheese was made with ordinary commercial rennet. The second cheese was made with crystalline rennin and the third with rennin plus impurities which are discarded during the preparation of crystalline rennin.

The crystalline rennin was made according to the method of Berridge & Woodward (2).

The impurities were prepared as follows; the filtrate obtained after saturating commercial rennet with sodium chloride was acidified to pH 1·8. A copious precipitate was formed. This precipitate was removed by filtration and stored in the refrigerator until needed. It had a low but definite activity, consequently when the amount of enzyme needed for 30 gal. of milk was calculated from the activity of the precipitate a much larger quantity of adventitious material than is present in commercial extracts accompanied the rennin. The crystalline enzyme was free from adventitious material.

Parallel experiments indicate that the acid precipitate contained about 10% of the active enzyme present in the parent solution and about 80% of the crude protein, making it about one-fifth of the specific activity of commercial rennet, on a dry-weight basis. The crystalline rennin had about ten times the specific activity of commercial extracts.

Both crystals and precipitate were prepared for addition to the milk by dissolving in dilute phosphate buffer of pH 6·8.

During making the control cheese behaved normally in every respect. The milk with crystalline rennin clotted very quickly (3 min.), but the curd remained soft for a relatively long time. In a small-scale parallel experiment similar curd was no firmer after half an hour than a curd produced by commercial rennet clotting in 9 min.

The curd formed by crystalline rennin developed less acidity than the control; on applying the hot iron test threads only $\frac{1}{4}$ in. long were formed in place of the normal $1\frac{1}{2}$ in. threads. After pressing, the acidity, though sufficient, was less than that in the control, and the curd was softer.

The curd formed with the preparation containing excess of adventitious material, when subjected to the hot iron test, formed a small number of threads which were both stouter and longer than normal.

When the cheese were tasted after ripening for 4 months, the control was of normal pleasant flavour. The cheese made with adventitious material had a similar but stronger,

i.e. more concentrated, flavour. This flavour was absent from the cheese made with crystalline rennin, and this cheese was in fact rather unpleasant.

If, as is generally assumed, the flavour is a product of the action of lactic acid bacteria, these results are in agreement with the hypothesis that impurities in the rennet stimulate, either desirable fermentations by the flora, or the growth of desirable strains. It is hoped to conduct further experiments in greater detail to obtain an insight into the changes that follow the use of different enzyme preparations.

I wish to thank Miss Helen Chapman for making the cheese and observing the properties of the curd, and Mrs C. Woodward for making the enzyme preparations.

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637.323.9

606. A VARIABILITY STUDY OF FIRMNESS IN CHEESE USING THE BALL-COMPRESSOR TEST

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INTRODUCTION AND DISCUSSION

It is generally appreciated that objective instrumental readings have advantages over subjective methods for determinations of physical properties in experimental and industrial processes, and in relation to cheese manufacture the problem has been discussed by a number of workers (Scott Blair (1), Baron (2), Wearmouth (3), Cox (4)). Cox (4) has pointed out that since the end-products of the fabrication process for cheese are highly variable units in respect of firmness, the essence of the problem in this case lies in the determination of a distribution or pattern of firmness readings which will characterize the immature cheese sufficiently to permit economical and reliable decisions as to future processes (storage for retail, etc.). The complete rationalization of cheese-testing processes would also necessitate the statistical investigation of an associated sampling problem arising from the fact that only a proportion of an output batch can be tested at one time. The modern approach to this would involve methods of statistical quality control, discussed in connexion with the dairy industry by Cox & Clarke (6). The present paper will, however, be restricted to the report of a controlled investigation into variation encountered with the ball-compression instrument for firmness determinations (Scott Blair & Coppen (5), Caffyn & Baron (7), Baron (2)), bearing directly on the basic problem defined above.

Part of the variability of an observation is contributed by the instrument itself and the process of using it, and ideally if this contribution could be isolated the remainder would reflect true variation in the material examined. The complex rheological properties of cheese, however (and in particular the fact that it is not truly elastic), makes it impossible to obtain exactly duplicate readings in any one place, so that the instrumental contribution cannot be obtained from observations on cheese itself. Variation due to the instrument alone, because of backlash, for example, reflects the accuracy of its construction, and in the case of the latest version of the ball compressor this contribution to the variation is likely to be small in practice (Wearmouth (8)). Variation arising from the process of using an instrument commonly appears, when observations are taken by a number of individuals, as observer to observer variation. This variation might merit experimental investigation, but experience has indicated that consistent results are obtained by different observers (Scott Blair (9), Wearmouth (8)). In the experiment reported here all the readings were made by one observer so that most of the variation encountered can reasonably be attributed to variability arising from real firmness differences in those cheeses tested.

EXPERIMENTAL PROCEDURE

In the spring of 1951 ten commercially made Cheddar cheeses, one from each vat of the production on a single day, were tested after a storage period during which they had been turned frequently to lessen differences between the two ends. All the ball-compressor

determinations were made through the rind on the upper face of the cheese; determinations at different levels had also been projected during the planning of the experiment, but in practice only a small-scale experiment for the variability study was found possible. Preliminary results had shown that there might be a firmness gradient on the face of the cheese from the circumference to the centre, so that it was decided to carry out tests at the centre and at points on concentric circles of radii 2 and 4 in. Consideration was also given to the possibility that proximate readings might not be independent in that the compression and incomplete recovery resulting from one test might transmit a strain pattern, so that neighbouring readings might show the cheese to be firmer than it otherwise would have been. To avoid this difficulty a minimum distance of 2 in. between neighbouring readings was prescribed, and subject to this restriction readings were taken at random positions on the circumferences of the two circles. There were three readings on the 2 in. circle and six readings on the 4 in. circle. As a further precaution, on five of the cheeses, selected at random, tests were made in the order—centre, 2 in. circle, 4 in. circle—and for the remaining five cheeses this order was reversed. The ball-compressor readings were recorded in terms of total deformation and percentage elasticity (Baron (10,2)).

RESULTS AND ANALYSIS

The mean values of the total deformation and percentage elasticity readings at the different radii are shown in Table 1; the first five cheeses are those for which the centre was tested first.

Table 1. *Mean total deformation and percentage elasticity readings across the upper faces of cheeses*

	Cheese no.										
	Centre tested first					Centre tested last					
	1	2	3	4	5	6	7	8	9	10	Mean
	Total deformation										
Centre	53·1	43·5	43·0	45·9	47·4	51·8	49·1	49·1	55·1	48·3	48·63
2 in. circle	53·8	44·2	40·9	43·9	47·2	50·6	45·9	49·1	51·5	48·8	47·59
4 in. circle	49·8	39·4	42·8	43·5	46·6	47·7	45·5	46·0	50·2	44·4	45·59
Mean	51·32	41·27	42·22	43·88	46·86	48·97	45·98	47·23	51·07	46·14	
	Percentage elasticity										
Centre	79·7	81·8	79·3	80·2	79·7	79·3	82·7	80·4	79·3	79·9	80·23
2 in. circle	79·8	81·4	80·1	80·5	80·1	80·3	82·4	80·4	80·2	79·4	80·46
4 in. circle	79·7	79·9	80·4	80·6	80·6	80·1	80·4	79·7	79·8	79·8	80·10
Mean	79·74	80·55	80·22	80·50	80·33	80·10	81·27	79·95	79·88	79·70	

Two things can easily be seen from Table 1 for the total deformation values: first that there were appreciable differences between the various cheeses, and secondly, that there is evidence of a firmness trend from high mean readings at the centre to low mean readings on the 4 in. circle, although not all the individual cheeses exhibit this trend. Neither of these two effects is shown by the percentage elasticity values. Further information has been obtained from the analyses of variance for the individual observations from which the tabular means were derived. These analyses and the results of significance tests are given in Table 2.

From Table 2 it may be seen that, for total deformation, the differences between cheeses were highly significant, indicating that the scheme of testing employed is fully adequate

for discriminating between different cheeses. In addition, the analysis for cheeses 6–10 indicated that the differences in mean firmness readings at various distances from the centre were also significant. No significant differences appear for the percentage elasticity values, and it appears that this observation is less sensitive than the ball-compressor deformation reading.

Table 2. *Analysis of variance of total deformation and percentage elasticity readings*

		Total deformation		Percentage elasticity	
		Cheeses 1–5	Cheeses 6–10	Cheeses 1–5	Cheeses 6–10
Item	D.F.	M.S.	M.S.	M.S.	M.S.
Cheeses	4	165·5930**	46·0607***	1·0457	3·9195
Circles	2	18·3270	50·8494**	0·1389	1·6412
Cheeses × circles	8	7·8718	2·9053	1·0221	1·2712

D.F. = degrees of freedom; M.S. = mean square. $**0{\cdot}001 < P < 0{\cdot}01$; $***P < 0{\cdot}001$.

Table 3. *Variance estimates (mean squares) of individual total deformation and percentage elasticity readings*

		Total deformation		Percentage elasticity	
	D.F.	Cheeses 1–5	Cheeses 6–10	Cheeses 1–5	Cheeses 6–10
2 in. circle	10	2·5180	2·7140	0·6420	0·6207
4 in. circle	25	2·2538	3·4257	1·0261	1·6862

D.F. = degrees of freedom.

Lastly, the estimated variability on the different circles may be considered. Appropriate values are set out in Table 3.

From Table 3 it can be seen that all the mean squares for the total deformation readings are in good agreement. These values may also be compared with the mean squares for the interaction of cheeses with circles in Table 2. The result indicates that for cheeses 1–5 differences in the firmness values at different testing radii were not consistent from cheese to cheese, and reference to Table 1 shows that the values for cheeses 1–3 did not decrease monotonically from the centre outwards. For the percentage elasticity Table 3 shows that the readings on the 4 in. circles were slightly more variable than those on the 2 in. circles, though the difference was not significant ($P > 0{\cdot}05$).

CONCLUSIONS

The first general conclusion from the present study is that the percentage elasticity readings were less sensitive for differentiating between cheeses than the more simply obtained total deformation readings. This agrees with previous practical experience.

Secondly, from the results for cheeses 6–10 there is the possibility that significant differences in firmness may exist across the face of the cheeses. The circles on the faces of members of the two cheese sets were tested in opposite orders to check the assumption that a minimum distance of 2 in. between readings was sufficient to avoid interference and, because significant differences were not shown by the results for cheeses 1–5, the rider that the above differences invalidate this assumption must be considered. It may be noted, however, that, as the testing moved from the outside to the centre, the total deformation

readings increased, that is, firmness decreased. This is directly opposite to an effect expected from the interference of successive tests. There remains the possibility that the ball compressor was sufficiently sensitive to detect a decrease in firmness caused by traces of fat exudation resulting from the tests. This cannot be entirely dismissed, but fat exudation would only be expected with cheeses of much lower quality than those examined (Wearmouth (8)). It may further be noted that even when the centre was tested first (cheeses 1–5) the readings indicated that it was generally less firm than the circumference, and altogether it seems reasonable to conclude that the existence of real firmness differences on the upper faces of cheeses has been demonstrated in this experiment. It is, of course, likely that, depending on process details of manufacture and storage, consistent firmness patterns may develop in other parts of cheeses not so far examined in controlled experiments. Any testing scheme which ignored consistent differences would lose efficiency unnecessarily and further investigation to confirm and extend the above findings is desirable.

Third, it has been shown that ten observations with the ball compressor, distributed as in this particular experiment, achieved a highly satisfactory discrimination between cheeses of different firmness. More strictly the discrimination was between cheese faces of different firmness since the lower faces of the cheeses were not examined, but all of the ten cheeses tested had received similar storage management prior to the experiment, so that it may reasonably be assumed that for discrimination, as distinct from descriptive purposes, the ten readings were adequate. Clearly much depends on the use envisaged for the firmness readings. If, for example, the future treatment of a cheese depends simply on whether or not its sufficiently characteristic firmness lies within or without a specified firmness range, then the detection of small differences may not be necessary. It is appropriate, therefore, to give preliminary consideration to the application of these experimental results to routine practical problems in cheese manufacturing. For this purpose a general estimate of the variation between ball-compressor total deformation readings at the same distance from the centre can be obtained as a standard deviation of 1·67 with 70 degrees of freedom. Using this value, 95% confidence intervals have been calculated for means of n ball-compressor total deformation observations, for various values of n. The results are given in Table 4.

Table 4. 95% *confidence intervals for means of* n *ball-compressor total deformation readings*

n	1	2	3	4	5	10
Interval	±21·2	±5·1	±3·1	±2·3	±1·9	±1·2

For practical purposes this may be interpreted as showing that if it were sufficient to determine an average cheese firmness to within ±2·3 units, for example, as measured by the mean ball-compressor total deformation reading, then four observations on the cheese would generally be adequate. This represents a slightly idealized approach, since the firmness contours have been assumed circular and differences between the two ends of a cheese have been ignored—in the sense that, by testing on both ends, such differences could be eliminated from between-cheese comparisons. The investigation of the importance of these differences has, however, been suggested earlier and it might lead to the conclusion that, provided all cheeses were treated similarly, some simple pattern for the readings

would suffice. In this case it seems likely that the figures in Table 4 give some approximation to the number, as distinct from the spatial distribution, of test readings required. Further, modifications of the original instrument can be envisaged to permit simultaneous multiple ball-compressor observations and to incorporate a single scale from which a value proportional to their mean might be read. Such developments might without great difficulty be arranged to give automatic readings on successive cheeses (Scott Blair (9)) and the process would be well adapted towards the economic organization of the testing scheme by the introduction of methods of statistical quality control for firmness.

SUMMARY

A variability study of ball-compressor readings on the upper faces of 10 Cheddar cheeses has shown that:

1. Percentage elasticity readings were generally less discriminating than total deformation readings.

2. As measured by the total deformation, there were firmness gradients across the faces of the cheeses, which tended to increase in firmness from the centre outwards.

Further experimentation to determine consistent firmness differences on cheeses has been recommended and meanwhile, from preliminary examination of firmness discrimination, it is suggested that four total deformation readings may give sufficient accuracy for many routine purposes.

Further information on the variability of ball-compressor readings is being prepared for publication by Dr W. G. Wearmouth. We are grateful to him and to Dr W. G. Scott Blair for helpful discussions and we wish to thank Miss Z. D. Hosking and Miss P. M. Clarke for technical assistance.

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REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION B: BACTERIOLOGY AND MYCOLOGY APPLIED TO DAIRYING

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Most of the new books published since the last Review (1) in this series are referred to subsequently under the appropriate headings; others of interest to dairy bacteriologists include a ninth edition of Mackie & McCartney's *Handbook of practical bacteriology* (2), a fifth edition of Frobisher's *Fundamentals of microbiology* (3), a fourth edition of Ainsworth & Bisby's *Dictionary of the fungi* (4), and Hobbs on *Food poisoning and food hygiene* (5). Volumes seven and eight of *Annual Review of Microbiology* (6, 7) were published in 1953 and 1954 respectively, and the third and fourth Symposia of the Society for General Microbiology on *Adaptation in micro-organisms* (8) and *Autotrophic micro-organisms* (9). A new journal published by the National Research Council of Canada appeared in August 1954; the *Canadian Journal of Microbiology* (10) covers a wide field of bacteriology, mycology and virology. The XIIIth International Dairy Congress (11) was held at The Hague in 1953, and the VIth International Congress of Microbiology (12) in Rome; six Symposia of the latter Congress have been published, on bacterial cytology, microbial metabolism, nutrition and growth factors, growth inhibition and chemotherapy, *Actinomycetales*, and the interaction of viruses and cells.

We record with great regret the death of Sir Alexander Fleming on 11 March 1955; also of Dr Leo F. Rettger, Professor of Bacteriology at Yale University, Connecticut, in January 1954, and of Professor Christian Barthel in July last year; Barthel's memoirs, published under the title *En mejeriforskare minns* (13), are fascinating reading of immense interest to all connected with dairying.

I. LACTIC ACID AND ALLIED BACTERIA

Our knowledge of the growth requirements of lactic acid bacteria has been reviewed extensively by Ritter (1), who has also contributed in this field, with Broquist and Nurmikko, to one of the Symposia (2) of the International Congress of Microbiology mentioned above. Sauberlich (3) has described a series of investigations on the nutritional requirements of lactic acid bacteria in synthetic media, while Kennedy, Speck & Aurand (4) have found a growth-stimulating factor, not associated with any of the known growth stimulants for lactic acid bacteria, in corn steep liquor. The stimulatory effect of autoclaved milk (115° C. for 15 min.) for some species of homofermentative lactic acid organisms (but not *Lactobacillus casei* or *Streptococcus liquefaciens*) was considered by Foster (5) to be due to partial hydrolysis of the casein, although excessive heating reduced the degree of stimulation, as shown by decreased acid formation in the milk cultures. Gibshman (6) has described acetic acid-producing bacteria resembling *Acetobacter aceti*, symbiotic with lactic acid bacteria, in sour-milk products; they were able to disintegrate milk protein and to utilize lactic acid and lactates. Studies of the alcohol tolerance of 1000 cultures of

various lactic acid organisms have shown, not nuexpectedly, that strains from wine and plants are the most resistant, and those from dairy products the least (7); alcohol concentrations exceeding 14–16% usually inhibited the latter.

Ecological studies in different regions on the Continent have suggested a relationship between the species of lactic acid bacteria found on plants and those present in dairy products typical of the different districts (8). Interesting observations from Japan (9) concern the species distribution of streptococci in starters, milk and milk products. Japanese butter cultures and spontaneously soured milk contained *Str. lactis* or strains intermediate between *Str. lactis* and *Str. cremoris*, dried milk and old mature cheese yielded *Str. bovis* and *Str. faecalis*, while *Str. cremoris* was isolated only from imported commercial powdered cultures. Garvie (10) describes a group of lactic streptococci which, although belonging to serological group N, were physiologically neither *Str. lactis* nor *Str. cremoris*, but resembled group D strains, *Str. bovis* in particular. Sachslehner (11) has attempted to differentiate *Str. lactis*, *Str. cremoris* and leuconostocs, chiefly *Leuconostoc citrovorum*, on morphological and physiological grounds. The limitations of morphological and biochemical criteria in the differentiation of *Str. lactis* and *Str. cremoris* have been discussed by Briggs & Newland (12), who showed, however, that these organisms are distinct entities serologically. An enrichment method based on competition with other lactic streptococci in milk has been described (13) for the isolation of *Str. citrophilus* (van Beynum & Pette); following a period of growth in milk the isolation is made on an agar medium containing potassium ferrocyanide and citrate in proportions in which the citrate inhibits the formation of prussian blue; decomposition of the citrate by *Str. citrophilus* results in the formation of prussian blue in its colonies. *Str. citrophilus* readily forms variants, some of which are described, though the physiological and serological identity of this organism with differently named citrate-fermenting strains in group N has been shown by Swartling (14) and Briggs (15). Swartling has given convincing reasons why this citrate-fermenting 'unit' should be accorded species rank under the name *Str. diacetilactis* (Matuszewski, Pijanowski & Supińska (16)). Clarke (17) has added phenolphthalein to the buffered medium used in the pH 9·6 test (18) for the differentiation of *Str. lactis* and *Str. faecalis*; the modified medium was easy to prepare and gave clear-cut results. Graudal (19) has described the cultural and biochemical characteristics of eighty-six strains of motile streptococci, chiefly from infants' faeces; all belonged to group D and about half were yellow-pigmented. The heat resistance of strains of *Str. lactis* and *Str. faecalis* has been studied by White (20) who found differences of value in distinguishing the two species. Nutritional studies of fifteen strains of *Str. thermophilus* have been described by Guss & Delwiche (21). In one of a series of papers on Indian *dahi* the taxonomy of the lactic acid bacteria concerned is described in detail (22); 430 strains of lactobacilli and 290 of streptococci were isolated from *dahi* and classified on morphology and cultural reactions; the lactobacilli were divided into three groups represented by *Lactobacillus bulgaricus*, *L. casei* and *L. brevis*, and the streptococci into four groups as *Str. thermophilus*, *Str. lactis*, *Str. faecalis* and *Str.* (*Leuconostoc*) *dextranicum*. Lactobacilli isolated from 561 samples of cream and milk, together with thirty-two known cultures, have been identified and classified by Edmondson & Jensen (23) using Tittsler's (24) and Briggs's (25) techniques; unfortunately, however, they gave no details of their results. Sharpe (26) has described a serological classification of the lactobacilli, based on precipitin tests, that closely parallels the physiological grouping of Briggs (25), and the latter has been extended (27,28). A special value of a new medium for lactobacilli (29) lies in its being inhibitory to species of *Bacillus*, *Micrococcus* and *Sarcina*, and a thallium acetate selective medium for lactobacilli has also been described (30). Wurtz (31) has cultivated a catalase-positive mutant of *L. plantarum*. Bhandari & Walker (32) isolated from brewery yeast a contaminating lactobacillus with a low optimum growth temperature ($< 30°$ C.) which they named *L. frigidus*; Russell & Walker (33) obtained from top-fermentation beer two strains of lactobacilli, also contaminants, which they considered, chiefly on the limited evidence of sugar fermentation reactions, to be further new species; they named them *L. parvus* and *L. malefermentans*. A review of the literature on lactobacilli with special reference to their taxonomy has been published by Briggs & Briggs (34), who observed 'much of the confusion in

the literature appears to have been caused by too much regard to names and sources of cultures, and too little to their stable and characteristic properties'.

In this Review *Starters*, *Bacteriophage*, *Inhibitory substances* and *Antibiotics* are considered from the more practical aspect elsewhere.

Attention to the freeze-drying of micro-organisms, including lactic acid bacteria, has continued during the period under review. Briggs, Tull, Newland & Briggs(35) have studied the preservation of lactobacilli by freeze-drying, paying particular regard to percentage survival rates; they obtained their best results in horse serum + 8% glucose suspending medium, and also good results in separated milk + 3% added lactose. With forty-four cultures representing all species of lactobacilli, the majority of their survival rates were over 50% immediately after drying and over 25% after 6 months' storage at room temperature in the dark. Freshly isolated strains also dried well, and satisfactory percentage survivals were still obtained after some years' storage. Physiological and serological studies(36) of lactobacillus cultures and antisera freeze-dried in this work showed that their characteristics were not affected by the technique used. Observations on the freeze-drying of starter cultures have come from America and Australia; Watts & Calbert(37) found 10% reconstituted milk to be a satisfactory freeze-drying medium, and that titratable acidities of 0·8% or lower did not affect the activity of the cultures after freeze-drying; Czulak & Hammond(38) showed that the activity of single-strain starter cultures of *Str. lactis*, *Str. cremoris* and *Str. diacetilactis* was preserved after freeze-drying and storage at room temperature for up to 16 months. They give a convenient method for reconstituting dried cultures in the cheese-factory laboratory, and show that reconstituted cultures react to the same bacteriophage races as before freeze-drying. Preservation of the bacteriophages of lactic acid streptococci by drying at 37° C. for 30–60 min. has been described by Prouty(39), who was able to recover them after 6 years.

Cowan(40) has discussed the maintenance of stock cultures of bacteria in general by freeze-drying and in nutrient media. *Biological applications of freezing and drying*(41), edited by R. J. C. Harris, contains articles by fifteen authors, the coverage including antibiotics, milk and other foodstuffs, media, viruses, bacteria and tissues. A *Directory and catalogue of micro-organisms maintained in Australia*(42) was published in 1952. A report of the papers and discussion at a meeting in London in 1953 arranged by the Permanent Committee of Culture Collections of Micro-organisms has been published by Her Majesty's Stationery Office under the title *A discussion on the maintenance of cultures by freeze-drying*(43). Although they freeze-dried strains of *Bacterium coli*, not lactic acid bacteria, the work of Record & Taylor(44) is particularly interesting; they found that 'the more dilute the suspension' (to be dried) 'the lower the percentage of organisms surviving.... The dependence of percentage survival on the concentration of organisms in the suspension to be dried was shown to be due to soluble material derived from the organisms themselves.' Such observations, with those of Prouty(39), may well point a way to modifications of technique and future studies of freeze-drying.

II. STARTERS

General

Lactic streptococci are exacting in their nutritional demands. Various amino-acids are necessary for growth, others are needed to initiate growth. Peptides and peptide-like substances are also important (Anderson & Elliker(1,2)). Nurmikko(3,4) has shown that certain strains, when growing together, are each able to synthesize vitamins or amino-acids essential for the growth of the other. For instance, *Lactobacillus arabinosus* and *Str. faecalis* are able to grow together in a synthetic medium lacking both phenylalanine and folic acid, although the former is unable to grow alone in the absence of phenylalanine, the latter in the absence of folic acid. Van der Zant & Nelson (5,6) found a proteinase and a peptidase in cell-free extracts of *Str. lactis*. Milk cultures incubated at 32° C. contained amino-acids and peptidases. Kizer & Speck(7) studied the response of *Str. lactis* and *Str. cremoris* to the addition of acetate and citrate to a nutrient medium. Both

species would grow in the absence of citrate if acetate was present in the medium, but while the inclusion of citrate in the medium improved the growth of *Str. lactis*, the growth of *Str. cremoris* was greatly reduced.

An important feature of butter starters is the ability to produce diacetyl. Modern starters are, therefore, usually mixtures of not only *Str. lactis* and *Str. cremoris* but also of *Str. diacetilactis* and/or *Leuconostoc citrovorum*. The diacetyl in milk cultures is produced chiefly from citric acid, but some strains of aroma bacteria are also able to utilize lactose for this purpose. Coppens & Antoine (8) found that strains of *Leuconostoc* could use lactose and Sachslehner (9) that 87·5 % of the strains of *Str. cremoris* isolated from cream starter cultures gave a strong Voges-Proskauer reaction. Similarly, a high proportion of the strains of lactic streptococci isolated by Zielinska & Hiscox (10) from Cheddar-cheese curd resembled type strains of *Str. diacetilactis* and *Str. citrophilus* in most respects, but differed from them in that they were able to produce acetoin (in relatively small quantities) from citrate-free lactose broth. According to Täufel & Krusen (11) strains of *Str. cremoris* did not produce acetoin from lactose in the absence of citric acid, but the addition of lactose to the citric acid medium increased acetoin production.

Russian workers have pointed out the loss of quality in starters that may result from the inclusion of strong acetoin-reducing strains. Maksimova (12) noted that some strains of *Str. diacetilactis*, though active producers of diacetyl reduced it rapidly, and if strong and weak reducing strains were combined in a starter no diacetyl remained in the culture at the end of 24 hr. Diacetyl production was reduced if *Str. lactis* or *Str. cremoris* were combined with *Str. diacetilactis*, but the culture was stable. If strains of *Leuc. citrovorum* or *Leuc. paracitrovorum* were combined with strains of *Str. diacetilactis* the diacetyl disappeared rapidly. This ill-effect of *Leuc. citrovorum* and *Leuc. paracitrovorum* on diacetyl production by *Str. diacetilactis* has also been commented on by Bogdanov (13) and Deryabina (14).

It is not possible by direct counting to determine the proportion of each type of organism in a mixed starter. Prouty & Glenn (15, 16) devised a medium on which the colonies of *Leuc. citrovorum* and *Str. cremoris* can be readily distinguished by shape and size. With further fortification of the medium *Str. cremoris* may be almost suppressed. In two mixed (*Str. cremoris* + *Leuc. citrovorum*) starters, the rate of growth was at a maximum during the first 13 hr. Acetoin and diacetyl could be detected for the first time at the end of this period, at pH 5·0.

Bacteriophage

Lactic bacteriophages are recognized as the cause of many of the cheese-making difficulties experienced in various parts of the world. Whitehead (17) has recently reviewed the position, drawing largely on his personal experience. It is well known that small amounts of phage in the cheese milk will disturb the normal course of acid development in the vat, but Czulak (18) observed that even large amounts did not affect subsequent acid production if the phage was added after the curd was cut. Nevertheless, phage increased in the whey and was also isolated from the cheese.

Airborne particles of phage from splashes of infected whey are known to be a potential danger in the cheese factory. They may be comparatively readily destroyed, by exposure to ultra-violet irradiation (19) or to aerosols of hypochlorites and quaternary ammonium compounds (20, 21). These measures are less effective against particles in thin films of milk or whey on floors or walls.

Phage-resistant starters. Not all the bacterial cells in a phage-infected culture are destroyed and normal growth is eventually resumed. By subjecting single-strain starter cultures to phage action for several generations Meanwell & Symons (22) obtained cultures resistant to that phage and often maintaining that resistance through several transfers in milk. Deryabina (23) obtained phage-resistant strains of *Str. diacetilactis* by incubating a phage-susceptible strain in milk in the presence of the phage, plating and picking colonies. A high percentage of the strains so isolated were phage resistant, and at the same time retained the property of diacetyl production.

It was mentioned in the last Review of this series (24) that bacteriophage did not proliferate in the absence of Ca in the medium, although this deficiency did not prevent adsorption of the

phage on the host cells. The amount of Ca necessary for growth varied with the strain—often very little was sufficient. Doull & Meanwell (25) cultivated lactic streptococci in 'Ca-free' whey or in a simple synthetic medium containing approximately 4 p.p.m. of Ca. Since the phage could not multiply, it was eliminated from the culture during repeated transfers. The studies of Potter & Nelson (26, 27) indicate that the Ca functions by promoting the invasion of the host cell after the adsorption of the phage has taken place. Its action is rapid and 38 sec. of contact between host, phage and Ca, or of action of Ca after adsorption, are sufficient to initiate proliferation of the phage. Mg, Sr, Ba would produce similar effects, but were less active than Ca in equivalent concentration.

Meanwell & Symons (22) and Doull & Meanwell (25) have shown that it is possible to maintain a supply of active phage-resistant and phage-free starters by storing them as freeze-dried cultures. These retained their activity during storage and, when revived in milk or the Ca-deficient medium, could be reintroduced into the cheese factory at suitable intervals. The use of the Ca-deficient medium for stock cultures and for subcultures preparatory to making bulk starters would protect the starters against the consequences of chance infection with phage.

Many chemical substances are inhibitory to lactic phages but, as Graham & Nelson's (28) experiments show, most of them are toxic to the host bacteria too. Crystal violet was, however, harmless to two strains of *Str. lactis* but inhibitory to their respective phages. It could be shown that the effect of the crystal violet was to extend the lag phase of phage development (subsequent development was at the normal rate), and that the yield of phage was closely related to the fraction of the latent period during which the crystal violet was allowed to act. The burst size of individual cells was much reduced.

Strain dominance. Starter cultures containing two or more phage-unrelated strains of lactic streptococci are often unstable. Mention was made in the last review of 'nascent' phage action, in which a non-susceptible strain is attacked by a heterologous phage when growing in mixed culture with a strain susceptible to the phage. Whitehead, East & McIntosh (29) have since shown that this is not a true lysis, but more in the nature of an inhibition due to a concentration of phage higher than normal. The mechanism of this inhibition is not yet clear, but it is not essential that the phage shall be 'nascent'; stock cultures were effective if the concentration of phage was high enough. Various strains of *Str. lactis* and *Str. cremoris* were susceptible to this inhibition, the latter particularly so.

Strain dominance in mixed-strain starters has been studied by Czulak & Hammond (30), who combined strains of *Str. cremoris*, *Str. lactis* and *Str. diacetilactis* in pairs and threes. The most stable mixtures were those containing each of the three types of organism, and such cultures could be maintained in an active state for long periods under factory conditions. Mixtures of strains of *Str. cremoris* were the least stable and sometimes one strain was dominant even after the first transfer. This dominance of one strain in mixtures of three unrelated strains was also observed by Lightbody & Meanwell (31). Of ten strains, three were outstanding and, within a few subcultures, dominated each of the three-strain cultures in which they were included. These strains produced substances inhibitory to the growth of other strains, though not at a rate sufficiently high to have a significant effect on acid production in the vat. There was no evidence of the production of antibiotics by the remaining seven strains. Cultures, consisting of pairs of these strains, could generally be carried through 20–60 subcultures before dominance in either strain significantly affected the susceptibility of the starter. Contrary to the experience of Czulak & Hammond, three of these stable cultures were pairs of strains of *Str. cremoris*.

An advantage of the use of mixed-strain starters is that a useful starter may develop even if the culture becomes infected with a phage active against one of the strains. If the infection is heavy enough to lyse a dominant strain quickly, a subsidiary strain may recover in time to produce a useful starter overnight. It is suggested this may explain the apparent immunity from phage trouble in some cheese factories where no effort is made to control phage infection. Any phage infection of the starter (generally mixed-strain) is likely to be heavy enough to lyse the susceptible strain quickly.

Typing of phages. Pette(32) has criticized the method of cross-reactions for the typing of phages and suggests that possibly the latent period and burst size could be used with greater effect. Although the latent period varies only slightly from strain to strain, there are wide variations in burst size, with a striking difference for some strains. Graham & Potter(33) also note that the length of the latent period and the burst size for three unrelated phage strains, each grown with their respective host bacteria, varied with the strain and the medium. They report that the latent period varied from 25 to 40 min. and the average burst size from 33 to 129.

In a study of six selected phage races isolated from cheese whey, Zehren & Whitehead(34) observed that the rate of development was related to the latent period and burst size, the faster the phage the shorter the latent period and/or the greater the burst size. The rate of development varied; in one phage race the maximum titre (which was of the same order for all races) was reached in 3–4 hr., in another the maximum was not reached in 7 hr. The latent period of the former race was the shortest for the whole group of six, that of the latter the longest. The burst size of the latter was the greater of the two, but the latent period was the more important factor in determining the rate of proliferation of the phage. This explained the varying susceptibility of starters in general factory use in New Zealand, some of which could be used for long periods, others for not more than 1–2 days in succession. Zehren & Whitehead suggest that a determination of the latent period and burst size of a phage isolated from cheese whey before signs of failure are apparent in the starter, may serve to predict the susceptibility of the starter and its probable useful life in the cheese factory.

The pioneer work on lactic phages was concerned with phages isolated during the course of Cheddar cheese-making. This type of cheese seems still to be the most seriously affected, but recent reports indicate that somewhat similar difficulties are experienced in the manufacture of Swiss type cheese. Deane, Nelson, Ryser & Carr(35) isolated a bacteriophage active against *Str. thermophilus* from a vat sample taken in a Swiss-cheese factory in the United States, where difficulties had arisen because of slow acid development. More recently Kiuru & Tybeck(36,37,38) have described a similar *Str. thermophilus* phage isolated from Emmental cheese made in Finland. The effect of these phages could be observed in the early stages of cheese-making, for example by low acidity in the whey at dipping and slow development of acidity in the cheese during the first 2–3 hr. in the press. The phages were specific for a single host strain and did not lyse other strains of *Str. thermophilus*, *Str. lactis* or *Str. faecalis*.

Kiuru & Tybeck report too the isolation from Emmental cheese of phages active against *Lactobacillus helveticus* and *L. lactis*. The presence of these phages sometimes became apparent during the first hours of pressing, but more frequently not until a later stage, when the pH of the cheese leaving the press was too high. The cheese did not drain well and were abnormally moist, with torn eyes and poor flavour. Phage-resistant strains of *Str. thermophilus* and *L. lactis* could be cultivated, but since their acid production was weakened in the process this was not a feasible method of control. The phages were not destroyed by H.T.S.T. or holder pasteurization or by the cooking in the vat, and they were not affected by 1 week's contact with a 25% solution of NaCl; active phages have been recovered from 6-month-old cheese. There are many similarities with other previously described lactic phages, and control measures would be on the same general lines.

Inhibitory substances

Raw and pasteurized milks are not always suitable for starter making or for the manufacture of cheese. This difficulty, which is seasonal, is widespread and the problem has been studied in England and Australia(39,40,41). The peculiarity is not confined to the milk of any one breed of cattle or to any particular area and is most in evidence in the winter. Not all strains of lactic streptococci are affected. The nature of the trouble suggests the presence of an inhibitory principle in the milk. Neither holder nor H.T.S.T. pasteurization removes the inhibition, but starters grow normally in the milk after it has been autoclaved. Czulak & Meanwell(39) suggest that milk contains a substance inhibitory to strains of *Str. lactis* and *Str. cremoris* and also a growth factor which, in summer, is present in an amount sufficient to counteract the effect of the inhibitory

substance. In winter the amount is small, and only those strains which can themselves synthesize this growth factor are able to withstand the inhibition. Jago (41) found that the inhibitory principle is associated with the fat globules and could be substantially removed from the milk by separation of the cream; the separator slime contains a heat-stable substance which stimulates acid production. The activity of a starter is related to the proportion of 'inhibited' to 'uninhibited' strains. The inhibition in cheese is most marked during the cheddaring process, but acid production could be boosted by the use of larger quantities of starter.

It is generally accepted that raw milk contains bactericidal or bacteriostatic substances. Mention was made in the last review of recent researches on this property of milk and of work in progress by Auclair & Hirsch (42) and Auclair & Berridge (43). This work was linked with researches on mastitis and was chiefly concerned with *Str. agalactiae*, *Str. pyogenes* and *Staphylococcus aureus*. In a later paper Auclair (44) has given data for *Str. lactis*. Of the two lactenins, L_2, found in milk, was strongly inhibitory to *Str. lactis*, but L_1, found chiefly in colostrum, was without effect. Acid production in sterilized-milk cultures of *Str. lactis* was inhibited when various concentrations of milk heated to 60 or 70° C., i.e. containing $L_1 + L_2$ and L_2 respectively, were added, but there was also a slight stimulatory effect in the lowest dilutions. The inhibitory substances were bacteriostatic rather than bactericidal. Cultures which showed no growth in 48 hr. could be revived by diluting out the inhibitory substances. They were not destroyed by either holder or H.T.S.T. pasteurization.

Some of the lactic acid bacteria produce substances inhibitory to the growth of other bacteria. One of the best known of these is nisin, produced by various strains of *Str. lactis*. This antibiotic is one of the causes of starter failure and slow acid development in cheese, but it also inhibits the growth of anaerobic spore-bearing bacteria, a property which has been used in suppressing butyric acid fermentation in Emmental and processed cheese. This question has recently been reviewed by Berridge & Mattick (45,46). Hirsch & Grinsted (47) have shown that the spores, especially those of *Clostridium butyricum*, are more susceptible to nisin than the young vegetative cells. Nisin is sporicidal for *Cl. butyricum*, but appears merely to inhibit germination of the spores of *Cl. sporogenes* and *Cl. bifermentans*. Gas production is more readily suppressed than growth. The lactate-fermenting strains of the butyric acid bacteria were less strongly inhibited than the lactose-fermenting strains (Sjöström & Willart (48)). Experiments by Storgårds (49) indicate that the inhibitory action of nisin (or the Seitz filtrate of a nisin-producing culture), added to a milk culture of a sensitive streptococcus, was considerably weakened if penicillinase were also added. Cheeseman & Berridge (50), on the other hand, found no evidence of such a restraining action by penicillinase.

Saccharomyces fragilis, a lactose-fermenting yeast isolated from gassy cheese, was inhibited by lactic acid bacteria (Stocker (51)). *Str. lactis* was the most active, many strains entirely inhibiting fermentation. Few strains of *Str. cremoris* but several of *Str. thermophilus* were active. *Lactobacillus bulgaricus* and *L. lactis* were also inhibitory. Winkler (52) reports the inhibition of eleven strains of propionic acid bacteria by a strain of *L. helveticus* and one of *L. acidophilus*. One antibiotic was heat-stable, the other heat-labile. Binder (53) found that eleven white and blue strains of *Penicillium camemberti* inhibited, with varying intensity, the growth of *Str. thermophilus* and *Bacterium linens*. The lactobacilli were only slightly affected and there was no inhibition of *Oidium lactis* (*Geotrichum candidum*). No similar inhibitory substances were produced by *Penicillium roqueforti*. Wilkowske & Krienke (54), however, found no substance inhibitory to the normal development of starters in extracts of three American brands of Camembert and four of Blue and Roquefort cheese. They concluded that *P. camemberti* and *P. roqueforti* did not produce antibiotic substances.

Antibiotics

The difficulties that have arisen in the cheese and butter-making industries as a result of the excretion of antibiotics in the milk after intramammary treatment of cows suffering from mastitis, and the measures taken to mitigate these difficulties are well known. The subject has been fully discussed in reviews by Overby (55) and Thomé (56). It is usual to prescribe a definite period during which milk from cows under intramammary treatment with antibiotics must not be sent to

factories for use in cheese-making. The Danish Dairy Research Institute (57) has recently pointed out that, with the use of antibiotics other than penicillin, longer periods than those now stipulated may be needed to ensure that the milk is free from antibiotics. There is also the danger that antibiotics injected intramuscularly into the cow in the treatment of diseases other than mastitis may pass into the milk via the blood. Sadek (58) states that when penicillin was injected intramuscularly into healthy cows the antibiotic was present in the milk 2 hr. afterwards and could still be detected at 24 hr. When injected into cows suffering from mastitis it was found in the milk in ½–1 hr. Overby (59) has reported that nebacetin, a mixture of neomycin and bacitracin, was less inhibitory to starter bacteria than most other antibiotics—20 μg. per 1 ml. milk were needed for complete inhibition—and was excreted in the milk for a shorter period after intramammary injection.

Penicillin-resistant starters. It is possible, by selection of colonies or repeated cultivation in the presence of antibiotics, to produce starters resistant to those antibiotics. Hietaranta & Timroth (60) found that greater resistance was attained in shorter time when autolysed yeast was added to the milk culture. The resistance acquired by *Str. lactis*, *Str. thermophilus*, *Str. faecalis* and *Lactobacillus helveticus* persisted, but that of *L. casei* was quickly lost unless autolysed yeast was added to the milk cultures. Unfortunately, these antibiotic-resistant cultures lost some of the activity of the parent strain. Weaker aroma production may bring serious problems to the butter-making factory. Overby (61) found it possible to produce a mixed-strain starter which was not only resistant to penicillin, but also maintained its aroma-producing capacity. Nielsen (62) found that a penicillin-resistant starter developed acidity more slowly than a normal starter with which it was compared, and less readily in penicillin-containing than in penicillin-free milk. Johns (63) found that the sensitivity of mixed-strain starters varied from starter to starter and from day to day in spite of efforts to maintain constant conditions. There was no correlation between the dominant species and the resistance of the starter. The proportions of *Str. lactis* in the more resistant, and of *Str. cremoris* in the more sensitive starters varied widely.

Detection of antibiotics in milk supplies. When a strain of *Str. lactis* is used to detect antibiotics in milk the result is not available for several hours, which is a serious drawback when cheese-milk is to be tested. Other strains, growing at higher temperatures, have lately been used to give quicker results. Ullberg (64) used *Lactobacillus bulgaricus*, incubated at 45° C. and observed the time of coagulation. He obtained results in 2½–3 hr. Treccani (65) also used *L. bulgaricus*. Berridge (66) used *Str. thermophilus*, which is sensitive to 0·01 i.u. of penicillin per ml. of milk. The culture was maintained in the logarithmic phase of growth in a milk + yeastrel glucose peptone broth medium. Brom-cresol purple was used to indicate acidity. The test samples were incubated at 45° C. and examined at ½-hourly intervals. A concentration of antibiotic of 0·06–0·015 i.u. per ml. could be detected at ½ hr. Hietaranta & Timroth (67) used *Str. thermophilus* as test organism, resazurin as indicator, incubated at 38° C. and examined at ½-hourly intervals. Results might be expected in ¾ hr. Fleischmann (68) used a Yoghurt culture, incubated the test culture at 44° C. for 2 hr. and compared its titratable acidity with that of a normal culture. With the aid of such tests it should be possible to decide within a reasonable time whether a milk supply is suitable for cheese-making.

III. CHEESE

Manufacture

Changing economic conditions created a need for a review of labour conditions in cheese factories. Regular and, if possible, shorter working hours were desirable, and in meeting these demands changes have been made in the routine of factories. A considerable amount of mechanization has been introduced and a shortening of cheese-making processes has been sought. Traditional methods of manufacture of Cheddar cheese do not readily lend themselves to mechanization—the cheddaring stage imposes a break in the process. In some of the suggested procedures a second cooking of the curd at a higher temperature has eliminated the need for packing and cheddaring

the curd. To ensure that an adequate number of active lactic streptococci survive the high scalding temperature a culture of a heat-tolerant, non-haemolytic strain of *Str. durans* is added with the usual starter culture. In a method which has been developed on a pilot-plant scale in the United States by Walter, Sadler, Malkames & Mitchell (1), the curd is cut and heated at 100° F. in the normal way. It is then pumped into a revolving drum, where about half of the whey is drained off, then into a kettle where it is heated to 115–120° F., and salted. The slurry of curd and whey is pumped into a new type of hoop, allowed to settle while standing in whey held at 115–120° F., pressed, waxed and ripened. Much of the process is mechanized, and the time from renneting to pressing has been reduced to 2½ hr. This has been made possible by the rapid acid production at the high temperature of the second scald. Similar methods have been tried in Canada (2).

A shortened process which does not differ so widely from the traditional method has been developed in New Zealand (3). Here also there is a second cooking and a culture of *Str. durans* supplements the normal lactic starter. The whey is drained off after a second cooking at 108–110° F. for 10 min. and the curd allowed to cheddar for 30 min. at 108–115° F., after which it is milled, salted, hooped and pressed. The whole process takes 3–3½ hr. In Australia (4) the process has been shortened by increased mechanization and by the use of rapid single-strain starters. Overcast, Jarman & Albrecht (5) point out that when *Str. cremoris* was predominant in a Cheddar-cheese starter the rate of acid production was higher in curd in which the temperature had been slowly raised (in 60 min.) from 86 to 98° F. than in curd which had been heated to 102° F. in 25 min. (*Str. cremoris* produces little acid at 102° F.), and the former curd was ready for milling 1 hr. before the latter. The time difference was only 25 min. when a single-strain culture of *Str. lactis* was used. The delay did not affect the final flavour score of the cheese.

A modification along quite different lines has been tried experimentally on a small scale by Whitmore (6, 7). Acidity was kept at a low level throughout the process by washing the curd with hot water, the immediate result of which was to decrease the lactose content. The cheese were similar to Cheddar cheese in some respects, in others they resembled Swiss cheese. Both normal Cheddar and Swiss cheese starters were tried.

Although raw milk is still widely used in European countries for the manufacture of Emmental cheese, pasteurized milk is used in the United States for the Swiss cheese made there. From a study of the heat resistance of sixty strains of lactobacilli, Bassett & Slatter (8) found that twenty-five of the strains were killed by heating at 165° F. for 18 sec., four by heating at 160° F. for 18 sec. and only a few by momentary heating at 150° F. If rather high pasteurizing temperatures were used, it might be advisable to increase the number of lactobacilli added with the starter. Control of pH during the manufacture of Swiss cheese is of great importance. The growth of *Propionibacterium shermanii* is greatly affected by changes in pH, especially within the range 4·7–5·5, and even slight variations in the pH during the pressing may affect the whole course of the ripening. The temperature and pH are of greater significance during the pressing than at any other stage. It is then that *Str. thermophilus* most actively produces the acidity which promotes the later growth of the lactobacilli. It is at this stage too that the effects of phages are most prominent (Tittsler & Sanders (9) and Tittsler, Wolk & Hargrove (10)).

A new cheese of the Roquefort type to which the name 'Nuworld' has been given has recently been introduced in the United States. A white mutant of *Penicillium roqueforti* is used and no blue veins develop in the ripe cheese. Its clean mushroom-like flavour is distinct from that of the Blue cheese. It is marketable in 90–120 days (Morris, Jezeski & Combs (11)). A process for preparing these white spores has been patented in the United States (12).

The maturation of Camembert cheese may be hastened and controlled by exposure to indirect ultra-violet irradiation during the first 7 days after manufacture. Yeasts are suppressed and *P. camemberti* can grow unhindered. In Kundrat's (13) experiments maximum mould growth was reached in 5 days in contrast to 10 days for non-irradiated cheese. The growth of *Lactobacillus casei* was hastened. Nelson & Winder (14) investigated equipment for accelerating the ripening of Cheddar cheese by means of ultrasonic waves.

The manufacture of cheese on a small commercial or laboratory scale is often necessary for

experimental purposes(15). Kleinert(16,17) has described a method in which dried whole milk of special quality is reconstituted and pasteurized. The cheese are made by the normal methods, but at all stages great care is taken to exclude contaminating organisms. He used such cheese to study the development of the lactic streptococci and lactobacilli in cheese. The most rapid growth occurred during the first 9 hr.

Microflora and ripening

A defect of increasing frequency in Cheddar cheese, in the early stages of ripening, is that which has been termed 'slit openness'. Two faults are implied, a development of CO_2 in excess of the quantity that can be absorbed by the curd and incomplete fusion of the curd particles. The pressure of accumulating gas tears the particles of cheese apart along adjacent surfaces. The causal bacteria are usually starter organisms. Czulak(18) traced the fault in Australian cheese to the presence of *Str. diacetilactis*, more rarely to *Leuc. citrovorum*. He comments on the frequency with which the former organism occurs in modern starters for cheese-making. Albrecht & Ashe(19) traced a similar fault in American Cheddar cheese to *Leuc. citrovorum* in the starter. This organism could be suppressed and the fault controlled by the addition of 1·7 % NaCl to the cheese or by incubating the starter at a temperature high enough to allow *Str. lactis* to dominate over the leuconostoc strains. The lower incidence of this fault in summer could not be explained by seasonal or lactational changes in the mineral content of the milk. Albrecht & Ashe comment that over-standardization of manufacturing procedure makes no allowance for adjustments to suit seasonal variations in the milk composition. A form of mechanical openness reported from Australia(20) is apparently the result of too short a mellowing time, which does not allow the curd to assume the correct physical condition.

Demeter, Janoschek & Günther(21) have amplified the data on the flora of Emmental cheese published at an earlier date. In general, the bacteria reach a maximum when the cheese is ready for pressing, with a decrease at salting and a slow decline throughout the ripening period. Until the salting the mesophilic streptococci, e.g. *Str. lactis*, are dominant; after that time the lactobacilli and later still the propionibacteria gain the ascendancy. The last are much in the minority at first, but after the salting they increase rapidly. Hietaranta & Antila(22,23) found that no more than traces of citric acid remained in commercial cheese of good texture and typical eye formation; in those with faulty texture appreciable amounts were left. The citric acid disappeared during the first week of ripening, most of it during the time of the most intensive growth of propionibacteria. This fermentation of the citric acid in Emmental cheese is due chiefly to the propionibacteria, but it is relatively unimportant for eye formation in comparison with the fermentation of lactates.

The ripening of Herrgård and Svecia cheese has been shown to be due chiefly to lactic acid bacteria(24). During the early stages the streptococci are dominant, but they decrease in numbers during the ripening process. The lactobacilli reach their maximum during the first 2 months and few remain in the ripe cheese. Propionibacteria are few in number and probably take little part in the ripening—propionic acid is not found in typical Herrgård cheese. Anaerobic spore-bearing bacteria are rare; butyric acid fermentation is not a common fault.

Unlike the parallel reaction in Emmental cheese, citric acid fermentation plays an important part in the ripening of Herrgård cheese. Thomé pointed out in 1949(25) that typical eye formation was linked with the presence of *Betacoccus* (*Leuconostoc*) *cremoris* in the cheese. Swartling & Lindgren(26) and Swartling & Mattsson(27) have carried the investigations further and have shown that *Str. diacetilactis* and *Leuc. citrovorum* are equally effective. The rate of the citric acid fermentation varies from cheese to cheese, but it is usually complete in 17–24 days. The first gas-holes appear at this stage. An effect of another nature has been cited by Järvik(28) in which a *Betacoccus* (*Leuconostoc*) sp. caused an abnormal fermentation in a Swedish cheese. Early gas formation, a somewhat similar defect, had been observed some years earlier by Galesloot(29) in farm and factory *Dutch* cheese. *Bc. arabinosus* and *Bc. bovis* were isolated from cheese and starter. Their effect was more marked if the lactic starter was slow and the fault was generally prevented if a really active starter was used. *Bc.* (*Leuconostoc*) *citrovorum* did not cause this defect.

A putrid smell which occurred intermittently in Gouda cheese was caused by *Bacillus polymyxa* (30), eventually isolated from the water supply. The same organism was isolated from Edam cheese, but the defect did not develop, probably because the high NaCl content of Edam cheese prevented the growth of the organism.

For more than a quarter of a century methods have been investigated for the manufacture, under United States conditions, of cheese of the Roquefort type, using cows' milk as a substitute for ewes' milk. The achievements, up to 1950, in the manufacture of American Blue cheese have been reviewed by Babel (31). Although Stilton cheese in many ways resemble those of the Roquefort type they differ from them in that a preliminary bacterial ripening precedes the mould ripening. Recently published figures allow a few comparisons of the two types. Hiscox, Harrison & Wolf (32) recorded changes in the volatile acids during the period of active mould growth; the water-soluble acids rapidly decreased and the fat-soluble began to increase. Nesbitt (33) notes a decrease in the volatile acids in American Blue cheese at 2–4 weeks, presumably during the development of the mould. He gives values of pH 5·2, total volatile acids 40·0 ml. 0·1 N-acid/100 g. of cheese and ketones 0·3–0·4 mg./5 g. of cheese for a well-ripened cheese. For Stiltons the corresponding figures were of the order of pH 5·9 and 30–36 ml. 0·1 N-acid/100 g. of cheese. Pre-war samples of Roquefort type cheese, bought in the English market, gave higher figures, e.g. 106–138 ml. 0·1 N-acid/100 g. of cheese and pH 5·2–5·7. For post-war samples the values were 30–50 ml. 0·1 N-acid/100 g. of cheese and pH 5·7–5·8. Berridge, Hiscox & Zielinska (34) demonstrated the presence of ketones in Stilton cheese, but obtained no reliable estimates of quantities.

The coat of a Stilton cheese, like that of a Blue cheese, is a bacteriological entity. Brindley (35) found that yeasts and lactobacilli or streptococci constitute the dominant flora of normal Stilton coats, and she suggests a sequence of streptococci and lactobacilli similar to that in the interior. Organisms other than the acid-producing types sometimes develop on pasteurized-milk cheese, and the yeasts decrease rapidly at the later stages. The scrapings from 'slip-coat' cheese contained *Proteus vulgaris*, *Str. liquefaciens* and *Oidium lactis*, all strongly proteolytic organisms not found on normal coats. The internal and external conditions that determine the type of coat, whether (normal) dry wrinkled or moist coloured, or (abnormal) 'slip-coat', are still undefined.

Experiments by Hartley & Jezeski (36) indicate how radically the slime on American Blue cheese may be affected by the ripening temperature. Cheese made according to the University of Minnesota method are ripened at 46–49° F. and *Bact. erythrogenes* predominates in the slime. *Bact. linens* predominates in the slime on the cheese made by the Iowa State method which includes ripening at 55–58° F.

The mixture of organisms, as described by Dommen (37), constituting the surface smear of Tilsit cheese from three Swiss factories, was the same for each factory and remained constant throughout the ripening period. Yeasts were outnumbered by bacteria. Non-pigmented non-liquefying rods predominated, but pigmented strains related to *Bact. linens* were there in significant numbers. They were able to break down the casein at the pH of the smear. The serological studies of Sands (38) indicate a relationship between *Bact. linens* and the genus *Corynebacterium*.

Flavour

The flavour of cheese remains elusive. Chromatography has given a clearer insight into the chemical changes which constitute the ripening processes, but has not yet enabled the scientists to establish the identity of the flavours characteristic of the various cheeses. Another approach to the problem has been an attempt to concentrate and identify the specific flavour substances. Such researches are in progress in England (39) and Australia (40).

In 1948 Dahlberg & Kosikowsky pointed out a connexion between the tyramine content of Cheddar cheese and its quality. Tyramine was not the flavour itself, but was an index of conditions in the cheese which promoted the flavour. Dacre (41) has examined a wide range of lactic acid bacteria but only *Lactobacillus brevis* was able to produce tyramine from tyrosine. He could find no good evidence of any significant connexion between tyramine and the typical flavour of

Cheddar cheese. An 'anty' flavour which sometimes occurred in cheese and other dairy products in Australia was identified by Forss (42) as methyl *n*-propyl and/or methyl *n*-amyl ketone.

Fatty acids. Since the researches of Peterson, Johnson & Price (43) it has been accepted that milk lipase is not active at the pH of cheese. Recently Albrecht & Jaynes (44) demonstrated that milk contains two lipase systems with optima at pH 5·4 and 6·3 in addition to that with an optimum pH 8·5. This reopens the possibility that milk lipases may play an active part in the ripening of raw-milk cheeses. Strongly lipolytic rennet pastes and glandular extracts of the calf, lamb or kid are traditional in the manufacture of Provolone cheese. On purification these enzymes lose much of their activity. A product is sought which is suitably active and also hygienically sound (45). Wilcox, Nelson & Wood (46) showed that the lipase of *Geotrichum candidum* (*Oidium lactis*) liberated only water-insoluble fatty acids from butterfat. The titratable acidity of the medium increased, but no steam-volatile acids could be detected. Irvine, Bullock & Sproule (47) tried this organism for cheese-making. The fatty acid pattern of Cheddar cheese made from pasteurized milk to which the mould had been added was similar to that of the control raw-milk cheese. Significant amounts of fatty acids (C_6 and higher) were present in the above cheese, but were lacking in those made from mould-free pasteurized milk.

Paper chromatography has been widely used to identify the volatile fatty acids in the steam distillates from acidified cheese-mush + $MgSO_4$. Sjöström & Willart (48) point out the apparent absence in most chromatograms of the higher fatty acids which would indicate that lipolysis had occurred in the cheese. They suspected incomplete extraction of the acids from the cheese and devised, for their own analyses, an ether-extraction method. With its use they were able to demonstrate caproic, caprylic, capric and higher acids in Swedish hard cheese.

Amino-acids. No striking progress has been made in the understanding of the part played by amino-acids in creating the typical cheese flavours. The amino-acid patterns have been studied for additional types of cheese and at intervals during the ripening period. Usually paper chromatography has been used, but Lindqvist, Storgårds & Göransson (49) and Storgårds & Lindqvist (50) have found electrophoresis a more promising tool for distinguishing between various cheese. The peptide diagrams were characteristic for Swiss, Herrgård, Emmental, Port du Salut, blue-veined and Camembert cheese. Chromatographic studies and biological assays of a variety of cheeses have been made by Kosikowsky & Dahlberg (51), Kiuru, Tybeck & Virtanen (52), Koloušek & Michalik (53), Clemens (54), Hintz, Slatter & Harper (55), Oswald (56), Svendsen & Jermstad (57), Jacquet & Lenoir (58), Jager (59), and Wilssens & de Vleeschauwer (60).

Most of the amino-acids, notably glutamic and aspartic acids and leucine, may be found in the cheese at an early stage, sometimes within a few hours of manufacture. Many of them increase in quantity throughout the ripening period, a few decrease. Kosikowsky & Dahlberg (51) point out the high proline content of cheese of the Emmental group. From the analysis of thirteen Swiss-type cheeses Hintz *et al.* (55) deduced that a minimum of 1·2% proline and 0·9% propionic acid was needed to give a medium intensity of the characteristic flavour. High concentrations of histidine were also found in cheese of good flavour; Kiuru *et al.* (52) found that, although appreciable amounts of arginine were present on the first day, it was not found after 45 days. Aspartic acid also disappeared after this time. Most amino-acids remained constant or decreased between the 40th–90th days, i.e. during the period of active development of propionic acid bacteria.

The amino-acids are of less importance than ammonium compounds in the ripening of Camembert cheese. Jacquet & Lenoir (58) found very small quantities of aspartic and glutamic acids and leucine in the cheeses at the 2nd day. More amino-acids could be detected on the 10th day, but proline was not found until the 18th day, when ammonia was first detected. The late appearance and meagre quantity of proline in Camembert cheese, also observed by Kosikowsky & Dahlberg (51), was in contrast to its early appearance and high concentration in Emmental cheese.

Of more direct microbiological interest are the implied decomposition of arginine and aspartic acid by propionic acid bacteria and the observation of Kristoffersen & Nelson (61) that serine, asparagine and threonine were deaminated by *Lactobacillus casei* at pH 8·1, but that only serine was decomposed at pH 5·4, with liberation of H_2S, a substance frequently found in Cheddar cheese.

Surface deterioration

Mite depredations on Cheddar cheese still constitute a serious problem and investigation of methods of control continues. Dicke, Ihde & Price (62) found that a 4% aqueous solution of sodium-*o*-phenylphenate + 0·5% of a gelatine spreader controlled the growth of eggs, resting and active forms of *Acarus siro* L. and *Tyrophagus castellanii* Hirst on slabs of cheese on which they had been cultivated. Dipping the cheese was more effective than spraying; the treatment needed to be repeated weekly. Wiping the cheese with mineral or cottonseed oil, or better still dipping in oil or glycerine was also an effective control. Robertson (63), whose researches into the habits of cheese mites and their control are well known, was impressed by the efficiency of the control in Dutch cheese-ripening rooms. To the meticulous cleanliness of these stores (Robertson's strong advocacy of careful scrubbing of shelves etc., as the most effective control, was mentioned in the last Review) is added frequent brushing and oiling of the cheese surfaces during the ripening period. The softer texture of the Dutch cheese, and the higher temperature and humidity of the ripening rooms, make them more susceptible than Cheddar cheese to mite infestation—yet this does not present a really serious problem in the Netherlands. The ripened cheese are waxed or sealed in a bladder envelope before transport, and Robertson suggests that cleaning of the cheese, as well as the shelves, and transport in mite-proof wrappings might help to solve the problem for Cheddar cheese.

Surface mould growth is controlled by treatment with fungicides, usually derivatives of propionic or benzoic acid. Sorbic acid has recently come into prominence for this purpose. It is not more effective than other fungicides, but it has the advantage that it does not suppress the growth of lactobacilli or leuconostocs, and, what is of greater importance, appears to be non-toxic (64, 65). Tentative approval for the use of sorbic acid-treated wrappers on cheese has been given by the United States Federal Food and Drug Administration (66).

The activity of sorbic acid depends on the undissociated portion, and pH is therefore the greatest controlling factor. Only 9% of the acid in a 0·1% solution is undissociated at pH 7·0, but 70% remains at pH 4·5. Even at pH 6·0 a considerable amount is in the undissociated state (67, 68). Thus sorbic acid is specially suitable for the treatment of cheese.

Sorbic acid rapidly passes from the wrapper into the cheese. Sometimes as little as 9·7% may remain on the wrapper at the 7th day after packing, and it may have penetrated $\frac{5}{8}$ in. into the cheese. The acid tends to disappear from wrapped cheese not by oxidation or escape through the wrapper but by the metabolic activity of the moulds (69, 70).

IV. BUTTER

Very little direct microbiological research on butter has been reported during 1953 and 1954. The efforts of the butter-making countries have been directed towards the manufacture of butter which, after deep-cold storage, will still be of good quality when distributed. The defects that occur during distribution are often microbiological. A fine dispersal of the serum droplets is a safeguard against the development of significant numbers of micro-organisms. The European countries have been the most active in the investigation of this problem. Two points that have emerged from their researches are, that butter may be cold stored equally well in small pats or in bulk provided the pats are sealed in air-tight wrappers, and that butter stored as small pats is of better quality on distribution than butter stored in bulk and made into pats on removal from the cold store. There is much evidence that, once the butter has 'set', the fine dispersal of the serum, such as that in the freshly worked butter, cannot be restored. 'Printing' directly after churning is advocated. References (1)–(10) give details of some of these researches.

McDowall, Singleton & O'Dea (11) have shown that the bacterial count and general quality of butter are not affected if the washing stage is omitted. A case of rancidity in butter was traced by Miklik (12) to the action of a Rhodotorula. Von Gavél (13) has suggested that a modification of the Little-Plate method should be used in the study of bacterial problems of butter. Quick

results are obtained, and the structure of the young colonies can be examined. Experiments by Bogdanov (14) and Blok & Geĭmberg (15) indicate that the addition of yeasts may prevent mould infections and other faults in butter.

In *The Butter Maker's Manual* McDowall (16) has collected a wealth of information covering the various aspects of the science and art of butter-making.

V. MILK CONTROL

Roeder's *Fundamentals of dairy science and technology* (1) contains 830 pages of interest in the field of milk control, though Parts I, III and V of the book deal more specifically with legislation, the microflora of milk and milk products and analytical techniques including bacteriological methods. The greater part of *Dairy microbiology*, by Dorner, Demont & Chavannes (2), covers the special bacteriology of milk, cream, butter, cheese and other milk products, and the tests used in their microbiological control. Demeter's book, *Bacteriological tests in the dairy industry* (3), describes the methods for the bacteriological examination of milk and its products, and is not confined to German practice and German literature; tests for pathogenic organisms are not included in the book. A tenth edition of the American *Standard methods for the examination of dairy products* (4) was published in 1953, and Clegg & Mattick (5) have reviewed recent research, particularly at the National Institute for Research in Dairying, on the bacteriological grading of raw milk.

In studies of the bacterial content of freshly drawn milk Agenjo (6) found that sterile milk could readily be obtained from goats and ewes, after washing and disinfecting the teats, but rarely from cows (7); micrococci predominated in the latter samples and Agenjo regards their presence as a distinct 'infection'; he emphasizes the importance of eliminating such infections from herds, since the organisms may not be destroyed by pasteurization. Murphy & Stuart (8) describe a technique for obtaining sterile samples of cows' milk by teat-wall puncture, using a syringe and needle. Demeter, Rau & Sack (9) have published evidence that the diet of the cow may influence the bacterial flora of her milk. The effects of high-speed centrifugation on the bacterial content of milk have been studied recently (10,11), the numbers of organisms being considerably reduced at speeds between 15,000 and 20,000 r.p.m. 90–95 % of the bacteria were removed from the milk, with little loss of milk solids, at rates varying according to the species, spore-bearers being removed the most rapidly, and *Bacterium coli*, *Proteus vulgaris* and *Pseudomonas fluorescens* relatively slowly; the rapid effect on spore-formers suggests that the technique may find practical application. Kästli & Binz (12) have shown that increases in the bacterial count of cleaned, empty milk cans, with the resultant effect on the keeping quality of milk, may be due to residual moisture in the cans; they emphasize also the dangers of inadequate cooling of the milk. In this connexion a paper from France (13) reviews the different methods available for milk cooling, while Kohn (14) obtained good results in sterilizing the atmosphere of a milk-cooling room by ultra-violet irradiation; when the ultra-violet lamps were operating continuously the bacterial count of the milk was reduced by 16·1 % during passage over an open cooler. Bacteriological deterioration of milk during transport and storage has been discussed by Jacquet (15) and Atherton (16), and Claydon (17) has shown that milk deteriorates more rapidly in summer than in winter, despite standardized inoculation and incubation procedures; the precise significance and application of this point is not yet clear. Claybaugh (18) has classified into four groups 239 strains of non-sporulating obligately anaerobic bacteria from dairy products, and Nashif & Nelson (19) showed that the majority of thirty-two strains of microbacteria which they isolated from milk and dairy products were strains of *Microbacterium lacticum*: they were unable to find *Micr. liquefaciens* in American products.

The heat-resistant bacteria able to survive pasteurization have been studied extensively of late, Allen (20) and Gyllenberg (21) reviewing the subject. Of particular value in this field are the contributions of Cuthbert, Thomas, Edgell, Nichols & Cox (22), Cuthbert, Edgell & Thomas (23), Thomas, Thomas & Ellison (24), and Thomas, Hobson & Griffiths (25), and of Bilimoria & Bhat (26), who identified sixty-two out of ninety-two strains of heat-resistant organisms from milk as *Bacillus*

species. It now seems generally accepted that 3 days' incubation at 30° C. is adequate for routine estimations of thermoduric organisms, and that these bacteria are prone to 'build-up' on milking equipment, especially that merely washed in hot water or detergent solution; the effect is prevented by steam or hypochlorite sterilization. Thermoduric contamination of milk cans from mechanical washers has been reported (27), and it has been shown (28) that the thermoduric count, in milk, is influenced by the time of year. According to Campbell (29) the temperature of growth of thermophiles may be influenced by their nutritional environment, the concept of variable heat resistance agreeing in part with the views of Bilimoria & Bhat (30,31), who maintain that thermophiles develop as heat mutants of mesophiles. A full account of recent work on psychrophilic organisms, of which *Pseudomonas* species are the most important in dairy products, appears in an able review of the microbiology of dairy products by Jezeski (32), who, with Greene (33), has studied the growth characteristics of these bacteria; with decreasing temperature (35–0°) the maximum viable count increased, but the growth rate decreased, so that at 0° the maximum count might not be reached for 10 days; the biochemical properties of the strains were altered at low temperatures. Nelson & Baker (34) recommend 3 days' incubation at 21 or 25° for the detection of psychrophiles. In general, psychrophilic bacteria are destroyed by pasteurization, their elimination depending largely on the cleanliness of the plant (35), but Lawton & Nelson (36) found that when cultures of psychrophiles were partially inactivated by heat before inoculation into skim milk, the lag phase of growth was greatly extended, with very rapid growth eventually during the logarithmic phase. Thus there seems to be a danger of late spoilage with occasional psychrophiles which may escape pasteurization.

Direct tests

The bluelight-fluorescence microscope has been shown recently to be of value in milk hygiene tests, as it can be used for the detection of various groups of micro-organisms, including pathogens, in milk; the techniques, outlined by Münchberg (37), differ according to the type of organism to be detected. Lämmler (38) has described in detail the technique for Mycobacteria and claims to differentiate *Mycobacterium tuberculosis* from the saprophytic species *M. phlei* and *M. lacticola*. For direct microscopic examinations of milk, improvements of Breed's method (39), Broadhurst & Paley's stain (40) and the methylene-blue stain (41) have been suggested, and Morgan & MacLeod (42) have improved their sequential scheme for grading raw milk on the basis of microscopic counts.

New milk-free agar media, approved in America (4) for the plating of milk and dairy products for official purposes, obviate the difficulties associated with media containing milk; the media were studied by Buchbinder, Baris & Goldstein (43) and Barber, Fram & Debaun (44), who found their productivity to be almost equal to that of media containing skim milk. Official procedures for the plate count of bacteria in milk have also been laid down in the Netherlands (45), and Schwarz, Ciblis & Fricker (46) have shown again that some of the variability in plate counts may be due to different brands or even batches of peptone. In studies of the various methods available for colony counts of milk George, Griffiths & Thomas (47) found that agar strips gave significantly lower results than plate counts and recommend their use only as a screening test; however, Higginbottom (48) obtained similar results with the two methods, and lower counts with roll tubes, and favours the agar-strip technique for routine purposes. Disparity of results between individual workers is further illustrated by the observations of Pritchard, Griffiths & Thomas (49) and Heinemann & Rohr (50) in America, in which roll tube or bottle counts compared favourably with plate counts, both for milk and for milk bottles. The limitations of swab tests for determining the bacterial content of dairy equipment have been demonstrated convincingly by Thomas, Griffiths, Elson & Bebbington (51); Wright (52) has evolved a method for the rapid determination of the logarithmic average of four plate counts.

The value and significance of *Bact. coli*, chiefly type I, as an indicator of faecal contamination has been questioned recently, Leininger & McCleskey (53), in water pollution studies, placing more reliance on the enterococcal count than on coliform counts. In this connexion Briggs and his colleagues (54,55,56,57) have shown that, contrary to general belief, coliform organisms do not

constitute the majority flora of the intestine and faeces; at any rate in the pig, chick and bovine (rumen) coliforms are far outnumbered by certain species of streptococci and lactobacilli. Substitution of lactobacilli for coliform bacteria in tests for faecal pollution would be inadvisable if only because of their exacting growth requirements, but streptococci could readily be identified both generically and specifically. Some authors (58, 59) have recently turned their attention to the enumeration of, for example, *Str. faecalis* in polluted waters. Ritter (60) has described the properties of coliform bacteria, using as examples 252 strains from milk, and the methods available for their detection and identification; a simple serological classification in the field of milk control is yet to be seen. The incidence, source and type distribution of coliforms in milk has been studied in some detail (61, 62, 63), and it has been recognized (64) that spore-bearers, especially in chlorinated water supplies, may be an important cause of false positives in presumptive coliform tests; colonies obtained in such tests should always be examined. The general tendency for milk samples with high standard plate counts also to have high coliform counts has been confirmed by Courtney (65). Evaluations of the different techniques for detecting and enumerating coliform bacteria in milk have been many, Kalshoven (66) preferring violet red-bile-agar plates, although with this medium Morris & Cerny (67) experienced difficulties with heavily contaminated milks. Salquist, Wallgren & Sjöström (68) have studied modifications of the nitrate-reduction test, and Murray (69) has shown the superiority of 30° incubation (over 37°). Triphenyltetrazolium chloride (TTC) agar has given good results in the determination of coliforms in milk (70, 71), but the most recent development involves the use of penicillin (72); according to Malling Olsen (73) this antibiotic is considerably more efficient as an inhibiting agent in selective coliform media than the basic dyes used at present.

The membrane filter technique is another important new development of great value in many bacteriological fields. In this technique (74, 75) organisms can be concentrated on a thin filter from aqueous solutions or gaseous substances, the filter then being placed on an absorbent pad with added nutrient broth. After 4–8 hr. incubation, for example, colonies can be observed with low-power magnification, and after 12 hr. can usually be seen with the naked eye. The method is particularly valuable where small numbers of bacteria are involved, and where the customary procedures would fail to detect them. Colonies are more readily seen in a modification of the technique (76) in which the filter is air-dried, mounted in immersion oil which renders it transparent, and examined under the phase-contrast microscope. Jannasch (77) has used the membrane filter with bacterial cultures in liquid media; the liquid culture, suitably diluted, and fixed with formalin, is drawn through the filter which is then stained, mounted and examined microscopically; this method, again, may be used for low concentrations of organisms. Uniformly good results have been obtained with the membrane filter technique in the examination of water and milk for coliform organisms (78, 79, 80, 81); the method was, in general, quicker and more sensitive, and gave equally good or better results than other techniques. An added advantage in water work was the fact that relatively large quantities of water could be examined with the minimum of time and materials (82). The filter can, of course, eventually be divided into portions for different procedures and examinations.

The superiority of guinea-pig inoculation over other methods for the detection of tubercle bacilli in milk, despite the development of a new technique for staining acid-fast bacteria (83), has been confirmed by Smith (84).

Indirect tests

Evaluations and comparisons of dye reduction tests for the control of the bacterial quality of milk have been numerous with methylene blue and resazurin, but a tetrazolium (triphenyltetrazolium chloride or bromide) reduction test has also been used to some extent. The test is easier to perform, and requires smaller quantities of milk (85) than the methylene-blue test, and in general gives equally good results (86), though it is perhaps not quite so selective for milk samples of intermediate quality. Detailed observations of the tetrazolium-reducing capacity of a wide variety of species of 'dairy' organisms have been made by Laxminarayana & Iya (87) and by Schwarz, Ciblis & Lange (88). The value of the catalase number (89) as an index of the degree of

contamination of both raw and pasteurized milk is considered by Caporali & Gevaudan (90) to be similar to that of the reductase test.

Modifications of technique for the methylene-blue test, and the influence of different factors on the results obtained with the test, have been described (91,92,93,94), and Barkworth (95) has correlated the results of plate counts and methylene-blue tests, showing the effect to be expected in one from a knowledge of the result of the other. Roeder (96,97,98) has made a particular study of the question of payment for milk according to quality as determined by reductase tests, which he considers would cause an improvement in hygienic conditions of milk production on the farm. The beneficial effects of such a system have been reported from Sweden (99). Tewes & Merta (100) have developed an improved methylene-blue test, incorporating rennet coagulation within 30–45 min., for milk paid for according to quality; they claim the method to be quicker than the original, and to avoid errors due to creaming. Considerable interest was aroused by the findings of Kelley & Dittmer (101) in America that administration of menadione (2-methyl-1, 4-naphthoquinone) to lactating cows, or its presence in *in vitro* tests, decreased the rate of bacterial growth in milk, retarding the souring of milk, and increasing the methylene-blue reduction time. However, the more recent results of Boyd (102), Clegg, Hoy, Chapman & Berridge (103) and Higginbottom (104) are not in agreement, since they found little or no effect of menadione either *in vivo* or *in vitro*.

The virtues of the resazurin test in routine milk grading have been emphasized by Hempler (105), and after detailed comparisons of four variations of the test Murphy & Grimes (106) have concluded that the 1 hr. test on samples directly after being taken is the most satisfactory for milk delivered at the creamery; the 10 min. method was suitable for platform rejections. Thomas (107) has presented extensive data on the use of the 10 min. test (and others) at creameries in Wales during 1945 and 1948, and a combination of 10 min. and 1 hr. resazurin tests has given good results in Austria (108). The resazurin test has also been used for detecting the presence of antibiotics in milk (109,110), though a titratable acidity test is more sensitive for this purpose despite the need for 6 hr. incubation periods in detecting low concentrations.

Defects

In recent work on 'broken' or 'bitty' cream in raw and pasteurized milk Rowlands (111) and Stone & Mattick (112) have shown that lecithinase-producing strains of the aerobic spore-formers *Bacillus cereus* and *B. mycoides* are responsible for the fault, the ultimate cause of the defect being breakdown of the fat-globule membrane by hydrolysis of lecithin. The researches into ropiness in milk made along similar lines by Jones (113,114,115) have now shown that ropy milk results from the breakdown of lactalbumin. The organisms incriminated were *Alkaligenes viscosus* and *Staphylococcus cremoris-viscosus*, whose characteristics have been described in detail (114); ropiness produced by *Bact. aerogenes*, however, appeared to be due to synthesis of a nucleo-protein. *Alk. viscosus* and *Bact. aerogenes* were most frequently encountered in Thomas's studies of ropy milk (116,117), in which water supplies were found to be the chief source of the organisms. Sundman's work (118,119) confirms that of Jones in part, since she demonstrated the protein character of a slime produced by strains of *Str. cremoris* from Finnish ropy sour milk, differing from the polysaccharide slime produced by *Str. bovis* and *Str. salivarius*; viscosity of the ropy milk depended on encapsulation of the *Str. cremoris* cells. Gainor & Wegemer (120) incriminated an encapsulated psychrophilic organism resembling *Alk. viscosus* in the development of ropy milk, the capsular material in this case being polysaccharide (121). Correlation of these observations and the further elucidation of this problem are awaited with interest.

VI. DETERGENTS AND DISINFECTANTS

A new technique for assessing the cleaning efficiency of detergents has been evolved by Mead & Pascoe (1) in Australia; clean microscope slides were 'soiled' in fresh whole milk at 98° F. for 15 min. and then subjected to detergent and rinsing treatment. The light transmitted through

them was measured photometrically, the difference before and after treatment being used as a basis for calculating the percentage cleaning efficiency of the compound; the method gave reproducible results, and the relative efficiencies of different concentrations of a number of detergents are compared. A test developed in America (2) uses organisms grown in a medium containing radio-active phosphorus (^{32}P) seeded on to the test surface. Percentage removal by the detergent of the test culture is calculated from the difference in radioactivity of the surface before and after cleaning. The principles and physico-chemical properties of detergents have been reviewed at some length by Pankhurst (3). Seelemann, Rackow, Pilz, Meyer & Schröder (4) and Seelemann, Rackow, Meyer & Schröder (5), in a series of papers on cleaning and disinfection on dairy farms, consider in particular cleaning and disinfection of dairy utensils, and of udders, teats and hands during milking; they compare British, American, Scandinavian and German practices and make recommendations for cleaning, chemical and, where appropriate, steam sterilizing, and storage of utensils. Extensive studies of the properties of detergents have been made by de Vleeschauwer, Hendrickx & Moulaert (6) and de Vleeschauwer, Wallez, Hendrickx & Naudts (7), who found, as Brauss (8) confirmed with disinfectants, that their efficiency was greatly affected by temperature; a lower concentration at a higher temperature was frequently more effective than the converse. Brauss found temperature differences between 10 and 35° C. to be most important, and rightly considers that the temperature at which an experiment is made should always be stated. It has been shown in laboratory trials, using an experimental recirculation cleaning unit and soiled stainless steel strips, that the addition of sodium hypochlorite solution (25–100 p.p.m.) to alkaline detergents assists in the removal of milk residues (9); the increased cleaning efficiency appeared to be due to the protein-dissolving action of hypochlorites. Methods for evaluating detergents used in can-washing machines have been suggested by Mohr, Jünger & Weinert (10); tests of a new type of can washer (600 per hr.) have been described by Plock & Falk (11), and studies of the cleaning of milk cans and tanks by Winther (12) and Zeilinger (13); using the swab rinse method, Winther found that a second swab 24 hr. after the first usually gave higher bacterial counts—this shows the importance of thoroughly drying cans after sterilization, a point confirmed by Mohr & Jünger (14). Tests of proprietary detergents have been made by Thomé, Nilsson, Signér & Nilsson (15,16) and by Jax, Futschik & Zeilinger (17), who discuss at length requirements in detergents for different purposes. Järvik (18) found in two detergent solutions of pH 11·3–11·7 an alkaliphilic flora consisting chiefly of spore-bearing rods.

Continued attention is being paid to the in-place cleaning of permanent milk pipelines; fully automatic electronically controlled equipment for this purpose is mentioned by White (19) in a general article on the subject. Stainless steel and glass are equally satisfactory materials (20), and there appears to be fair agreement now that these are best cleaned by chlorine solution preceded by hot-water washing or an alkaline detergent (21,22,23,24); the last two papers deal specifically with glass lines. Sheuring & Folds (24) have also studied the effect on cleaning efficiency of the flow velocity of the washing solutions; they concluded that at a velocity of 3 ft./sec. either hot water (190° F.) or chlorine solution (200 p.p.m.) satisfactorily sterilized permanent glass pipelines, destroying both coliform organisms and thermophiles. Studies of bottle washing by Resuggan (25) point again to the necessity for efficient physical cleaning of the bottles by detergents, and also to the vital importance of the final rinse water which should be bacteriologically satisfactory and adequate in volume. A new bottle-washing machine has been tested by Demeter (26), who made bacterial counts of the washed bottles by coating their inner surfaces with a thin layer of nutrient agar. Mohr, Jünger & Weinert (27) used a laboratory apparatus simulating a full-sized bottle-washing machine for evaluating detergents, and have shown (28) that the type of milk or cream residue left in the bottles significantly affects the number of bottles that can be satisfactorily cleaned by a given quantity of washing solution; the number decreased when the strength of the solution was lowered, but did *not* increase with increasing concentration above the particular appropriate value; this is a valuable observation of practical importance. They also found that the wetting ability of a detergent was completely lost during 7 months' storage in a dry, dark, well-ventilated room.

Quaternary ammonium compounds have continued in popularity, and Conklin (29) has evolved a simple field test for ascertaining quickly whether a solution is of adequate strength; different colour indications are given at concentrations either side of 200 p.p.m. Resuggan (30) has also developed a quantitative field test for quaternary ammonium compounds and has contributed (31), with Berry (32), Allen & Brooks (33), and Maurice (34), to a Symposium of the Society for Applied Bacteriology on disinfectants. Three detergent-sterilizers containing quaternary ammonium compounds were tested by Humphreys & Johns (35) by means of the glass slide and emulsion techniques. Each combination was more effective than its quaternary ammonium compound component alone, and hard water and low temperature depressed their activity; the necessity of using a quaternary ammonium compound neutralizer in plating techniques is pointed out. Futschik & Sachslehner (36) have examined in detail the properties of an aklyl-dimethyl-benzyl ammonium chloride, while Wilson (37) gives details of a colour test for the detection of quaternary ammonium compounds in milk and water. In studies of the disinfection of milk cans with sodium hypochlorite Hoy & Neave (38) found that the final bacteriological condition of a can was less affected by the concentration or method of application of the disinfectant than by rust or film on its inner surface; this points to the importance and even the economy of replacing faulty dairy equipment. 'Antibac' (39), a relatively new chlorine-liberating sterilizer designed to act in the pH range 5·8–7·0, has been used by Bacon, Sotier & Roth (40) on thirteen dairy farms in concentrations yielding 100–200 p.p.m. of available chlorine; successful results were obtained, and it is stated that the slightly acid nature of the compound minimizes hard-water deposits and helps in the removal of existing deposits and milk stone. Williams, Hirch & Cowan (41) have described a group of aerobic, Gram-positive cocci, to which they have given the generic name *Aerococcus*, prevalent in the air and dust of occupied places; similar organisms, with an extreme resistance to hypochlorites, have been isolated from dairy equipment. Coagulase-positive and coagulase-negative strains of *Staphylococcus aureus* have been found to be equally susceptible to hypochlorites and, with these organisms, it has been confirmed that the presence of protein (skim milk in this instance) reduces the efficacy of hypochlorite disinfectants (42), as is well recognized with quaternary ammonium compounds. Johns (43,44) has obtained good experimental results with iodophor compounds as dairy disinfectants, and Lindgren (45) with 'Tego 51', a surface-active disinfectant, developed in Germany, belonging to the ampholyte soap group.

The disinfection of milking machines is dealt with here as a separate problem requiring somewhat specialized treatment, partly because of all-important rubber components. Clegg & Hoy (46) lay particular emphasis on the treatment of rubber parts in describing methods of cleaning and sterilizing milking machines, and the importance of avoiding air locks when dipping teat-cups into disinfectant solutions has also been shown (47). Claydon (48) and Posthumus (49) show by comparative bacterial counts the advisability of using only teat-cup liners in good condition, and in the experiments of Cuthbert, Bird & Bateson (50) on chemical disinfection of an auto-recorder milking machine the liners were periodically defatted in 5% caustic soda. An interesting article by Bruggink (51) describes the work of The Central Committee for Milking Machines, set up in the Netherlands soon after the Second World War to advise on the use and maintenance of milking machines, including cleaning and sterilization. Wet storage of milking machine clusters, with periodic additional treatment, appears perfectly satisfactory (52), and wet-stored liners have a longer life than those sterilized by steam (53). Ezzard & Bennett (54) considered hypochlorite to be better than lye solutions for wet storage, but favoured quaternary ammonium compounds for cleansing liners by momentary dipping between individual milkings. As judged by rinse or milk counts Mallman, Peabody & Muentener (55) obtained better results, in general milking-machine disinfection, with hypochlorites than with quaternary ammonium compounds, and Bratlie (56) found the activity of quaternary ammonium compounds, particularly against coliform organisms, was reduced in the presence of protein to a greater extent than that of chlorine sterilizers. Eisenreich, Becker & Tewes (57) also preferred chlorine to detergent sterilizers (or hydrogen peroxide); they, like Claydon (58), obtained samples for bacteriological study by drawing sterile fluids through the milking apparatus. Some preference seems evident for chlorine preparations

for most aspects of milking-machine hygiene, and these are quicker-acting than, for example, quaternary ammonium compounds (55); however, there is still no unanimity of opinion. Krenn & Binder (59), in Austria, have tested *in vitro* a formalin preparation used for udder disinfection against *Str. lactis*, *Str. agalactiae* and *Bacterium coli*; 'Formulsin' was considerably less effective against *Bact. coli* than the other two organisms, but it is noted that the activity of pure formalin against *Bact. coli* was enhanced by the addition of a wetting agent.

VII. PASTEURIZATION

In his excellent review on the microbiology of dairy products Jezeski (1) has dealt at some length with the subject of pasteurization, with particular regard to the fate of pathogenic micro-organisms. The new books in this field include a comprehensive and well-illustrated monograph, *Milk pasteurization: planning, plant, operation and control*, by Kay, Cuttell, Hall, Mattick & Rowlands (2), Davis's *Pasteurizing plant manual* (3) and Atkinson's *Handbook of L.T.P.H. (low temperature positive holding) milk pasteurization methods* (4). *Milk industries* by Goded Y Mur (5) contains chapters on pasteurization, sterilization and irradiation of milk, and on dried milk and ice cream, with emphasis on practical aspects.

The continued popularity of high-temperature short-time pasteurization is reflected in the volume of recent work on the control of this process; the subject has been reviewed by Thomas (6). Davis (7) has also given an account of the control methods available, which generally involve the injection into the milk stream of salt, dyes, various chemicals, bacteria or radioactive isotopes. He has described a technique that, unlike those, can be used during a normal pasteurization run; hot milk is injected and its passage through the apparatus followed by means of the temperature changes which result; details of the necessary delicate recording instruments are given. Along similar lines Jordan & Holland (8) have studied the 'holding time' in H.T.S.T. pasteurizers by measuring, with an electronic timer, the time in which thermal waves, produced by manipulation of the steam supply, passed through the holder. Burton & Thiel (9) have discussed the significance of the holding time in H.T.S.T. pasteurization of milk, dealing particularly, like Posthumus (10), with the sensitivity of holding-time determinations. The 'chopper bar' temperature recorder studied by Plock & Lang (11) consists of a vapour-tension thermometer, fitted with a temperature indicator-recorder, the arm of which actuates the flow-diversion valve; it has been recommended as suitable for the control of milk pasteurizers (12). Cambosu's ammonium molybdate test has been recommended for routine use in milk pasteurization control (13), and the peroxidase (Storch's) test (14) has been studied extensively by Schwarz & Lange (15), who obtained relatively poor agreement in comparisons with the phosphatase test (Scharer & Black (16) modified).

The present status of the phosphatase test and its recent modifications has been summed up by Gilcreas (17), and Davis (18) emphasizes the need for precautions in carrying out the test in view of the serious implications of positive results in pasteurized milk. The danger of reactivation of milk phosphatase after heat treatment, of which Wright & Tramer (19,20,21) have made a special study, must also be recognized, and reactivation of peroxidase has also been encountered (22,23). Various comparisons of the different phosphatase test techniques have been made (24,25,26), some specifically in connexion with the 'international phosphatase unit', yielding correction factors for the separate methods so that all results may be expressed as international units (27,28). Aschaffenburg (29) has described a simplified buffer, which can be supplied to dairy laboratories in solid form, for the Aschaffenburg-Mullen test. General studies of phosphatase inactivation in milk have been made by Hansen, Wood & Thornton (30), and a method of phosphatase determination based on the liberation of phosphate from sodium β-glycerophosphate is claimed by Saingt & Jacquet (31) to detect 1% of raw milk in holder-pasteurized and 3–5% in H.T.S.T.-pasteurized milk. It has also been suggested (32) that the effect of pasteurization on the stability of phosphates might be used to differentiate raw from heated milk, but the claim has yet to be substantiated.

The problems of cream processing have been discussed by Crossley (33) and, in particular, the pasteurization of cream, by Crossley & Cuttell (34). The last-named author has also described

a new method of pasteurizing and deodorizing cream for butter-making (35). Swartling (36) has studied the pasteurization of cream, using the peroxidase and phosphatase tests, and Hooley (37) with special reference to the effects of homogenization, ageing and viscosity; he obtained his best results with low-fat cream by homogenizing at 115–120° F. followed by pasteurization at 175° F. for 15 sec. Reactivation of phosphatase in H.T.S.T. and Vacreator-pasteurized cream has been recorded, but not after holder treatment (38).

The present trend in the construction of heat-exchangers in Germany is towards H.T.S.T. pasteurizers with pressed stainless steel plates; Hartig (39) has given an account of such apparatus and controlling equipment, and Lancelot (40) has discussed pasteurizing plant in general including the Vacreator. Another paper details the basic conditions required to ensure efficient operation of pasteurizers and illustrates many of the individual components (41). Workers in America (42) have studied the possibility of increasing the pasteurization temperature to a point where the time required to heat the milk to temperature is sufficient to ensure adequate pasteurization, thus eliminating the holding time required in normal H.T.S.T. pasteurization; preliminary observations with *Bact. coli* and phosphatase tests, and of flavour, gave encouraging results, and if further studies with thermoduric and pathogenic organisms are satisfactory existing H.T.S.T. equipment could be modified very simply. Rüters (43) has proposed a combination of flash and H.T.S.T. pasteurization in one plant, and in a new French method pre-heated milk (50–85° C.) is brought instantly to the pasteurizing or sterilizing temperature (70–140° C.) by direct contact with steam (44). Lang (45) has reviewed the technique of 'Uperization' in the manufacture of dairy products, the methylene-blue test at 100° C. being recommended for distinguishing uperized milk (46); reduction times (20–40 min.) were intermediate between those of H.T.S.T.-pasteurized and sterilized milk. Further studies of the bacteriological aspects of in-bottle pasteurization have been made by Clegg & Lomax (47), who discuss in some detail the use of heating and cooling curves in assessing the heat equivalents of the process. On the subject of cream line in pasteurized milk Hansen, Wood & Thornton (48) found that creaming was measurably impaired by treatment at 155·8° F. for 15 sec., and concluded that pasteurization sufficient to inactivate phosphatase necessarily reduced the cream volume.

Studies of the keeping quality of pasteurized milk, and of the bacterial changes which take place on storage have been many (49,50,51,52,53,54,55) and include data too extensive for individual comment; however, the flora of milk taken directly from the cooling sections of H.T.S.T. pasteurizers consists in general terms of microbacteria and micrococci, with thermoduric streptococci (56,57), which are usually replaced, sometimes very quickly, by aerobic bacilli, lactic streptococci, and occasionally psychrophilic organisms (58,59,60,61). Swartling (62,63) has discussed the possible origins and significance of some of these organisms. Buttiaux & Beerens (64) in France found 16% of samples of pasteurized milk to contain *Clostridium welchii* spores, usually 50–200 per litre; such concentrations can cause food poisoning in infants. They detected *Cl. welchii* contamination by means of glucose agar containing sodium sulphite and ferric alum on which the colonies were surrounded by large black rings.

In attempting to decide the time-temperature combinations required for the degree of destruction inferred in a specific pasteurization or sterilization process, a fuller knowledge of the thermal death-rates of various micro-organisms would be invaluable; Prouty (65) has discussed at length the definition and significance of thermal death time and thermal destruction curves and has referred to apparatus specifically designed to determine thermal death times of bacteria at high temperatures (220–270° F.). Tischer & Hurwicz (66) have discussed the thermal death theory from the mathematical standpoint, and thermal death time studies of *Brucella abortus* in milk have been published (67); pasteurization standards of 143° F. for 30 min. and 161° F. for 15 sec. allow safety margins of 26 min. and 12 sec. respectively. The importance of coliform as well as total bacterial counts in the control of pasteurized milk has been emphasized by Torre (68), and by Piraux, Lacrosse & Querton (69), who considered the coliform count more important than the thermoduric count in relation to keeping quality. Comment on the relative significance of coliform organisms in milk as indicators of faecal contamination has been made previously (p. 405).

Torre examined 200 phosphatase-negative, coliform-positive milks, finding *Bact. aerogenes* predominant in 52% of the samples and *Bact. coli* in 45%; however, in Walter & Christian's (70) experiments the majority of 150 coliform organisms from pasteurized milk and cream were Intermediate types. Petersen (71) prefers desoxycholate agar to the gentian violet method for the detection of coliforms, chiefly because results obtained with the latter erred on the side of leniency; the sensitivity of the nitrate-reduction test has been increased (72). Preliminary trials of the membrane filter technique (73) (see p. 406) have shown it to be satisfactory for the determination of coliform organisms in pasteurized milk, good agreement being obtained with the presumptive coli test.

The results of numerous experiments on the destruction of *Mycobacterium tuberculosis* in milk by laboratory and commercial H.T.S.T. and flash pasteurization have been presented by Seelemann, Rackow, Börger, Meyer & Schröder (74), who reiterate that destruction of the tubercle bacillus is complete in phosphatase- or peroxidase-negative milk; nevertheless, occasional positives may still occur, particularly with plant inefficiently run and/or lacking suitable control apparatus (75). Davis (76) has compiled useful and interesting data on the incidence of *M. tuberculosis* in the raw and heat-treated milk supplies of Great Britain, and Bundesen, Danforth, Woolley & Lehner (77) have studied the thermal destruction of the bovine-type tubercle bacillus in artificially infected chocolate milk; time-temperature combinations of 160–189° F./15–30 sec. were satisfactory. In a comprehensive review of the epidemiology of 'Q' fever Derrick (78) concludes that, despite the efficacy of pasteurization, when it is practised, prevention of human 'Q' fever (as of brucellosis) depends essentially on the control of the disease in animals.

Other processes

Recent developments in the processing of milk for liquid consumption, including most of the methods mentioned below, have been reviewed by Mann (79), and Lang (80) has written a detailed illustrated account of 'Uperization', the Swiss milk sterilization process, dealing also with work on the bacteriological and chemical quality of the product. The different methods of milk sterilization, and the relative merits and demerits of sterilized milk have been discussed by Kästli (81) and others (82, 83, 84), and Schulz (85) has written a well-illustrated book on the subject, giving many references. In practical studies of milk sterilization Burton, Akam, Thiel, Grinsted & Clegg (86) found that heat treatment at 100° C. for 90 min. did not give sufficiently low spore counts; 113°/35 min. reduced the thermophile spoilage to 29% (cf. *c.* 100%) and gave milk free from mesophile spoilage after 10 weeks; similar results have been reported by Ridgway (87). Concerning tests of sterilized milk for bacteriological quality, aerobic spore-bearers require very low oxygen partial pressures to retard the germination and growth of spores; 'commercial sterility' is therefore inadequate for milk and milk products and tests must be stringent; Mossel & Drion (88) have described their tests and conditions, and Fellows (89) has described a ferricyanide test for sterilized milk. Studies of ultra high-temperature (275°F./2 sec.) treatment of milk have been made by Cuttell (90), who experienced considerable difficulty in removing milk deposits from the plates of the apparatus. Supersonic (91, 92), electrical (93) and 'ultra-violet' (94, 95, 96) sterilization of milk have been the subjects of further inquiry recently and a new process for high-temperature treatment of milk has been described using a 1 sec. exposure to infra-red rays (97). It is claimed that heating element temperatures up to 1350° F. were used and that burning-on does not take place since there is no metal-to-milk heat transfer surface; the temperature of the milk leaving the unit is stated to be below boiling-point, and bacteriological data show that the product is not sterile. Electronic sterilization has been referred to by various authors (98, 99, 100, 101), Duffey (102) giving a table of radiation dosages required for sterilizing many foods including liquid milk.

In work on the growth-inhibiting effect of compressed oxygen on the milk flora Lück (103, 104) has shown that deterioration of the milk after treatment is due largely to *Str. lactis* which is more resistant to high oxygen tension than most other organisms of the milk flora, e.g. aerobic spore-formers; oxygen treatment was least effective at temperatures between 20 and 38° C., when

bacterial growth was only delayed, but above or below this range the effect became bactericidal. Wiser & Wiser (105) have also described a technique for the treatment of hot milk or cream by spraying in an atmosphere of oxygen. The destruction by hydrogen peroxide of *Brucellae* in milk, and of *Rickettsia burneti* in milk and cheese has been reported (106, 107), and it has been shown that the addition of methyl-*p*-oxybenzoate markedly increases the action of H_2O_2, especially at 12–14° C., although alone it is ineffective (108). A test suitable for field use which will detect 3–6 p.p.m. of H_2O_2 in milk has been described by Pien, Désirant & Lafontaine (109).

Little work on the bacteriology of dried-milk powder has appeared during the last two years, though Higginbottom (110) has continued her studies of the effect of atmospheric R.H. on the rate of destruction of the microflora of dried milk; the maximum survival of micrococci and streptococci occurred on storage at R.H. 5–20%, but the spores of *Bacillus subtilis* were extremely sensitive to dryness. Mohr & Koenen (111) in Germany have proposed a standard of 50,000 organisms/g. maximum instead of the current, 10,000–1,000,000/ml. reconstituted milk. The dried-milk, and other, processing techniques have been reviewed by Dermine (112).

Ice cream

A useful and well-illustrated booklet by Jørgensen (113) describes the micro-organisms which may contaminate ice-cream, their control, and the cleaning and sterilization of ice-cream manufacturing apparatus; Martin (114) has reviewed recent American research work on ice-cream. A modern plant for high-temperature short-time pasteurization of ice-cream mix has been described by Brissenden (115), and an H.T.S.T. plant has been developed in America which can pasteurize ice-cream at a rate of 300 gal./hr. or milk at 600 gal./hr. (116). Conditions for H.T.S.T. pasteurization of ice-cream mix, equivalent to 155° F./30 min., were determined by Speck, Grosche, Lucas & Hankin (117), using as test organisms *Microbacterium* and *Micrococcus* species, who detail the time-temperature combinations required for the destruction of these organisms. Work of similar quality on *M. tuberculosis* would be of great value, and the dangers of using heat-resistant substitutes for the tubercle bacillus in fundamental work of this nature have been referred to in a previous Review (118) in this series. Speck *et al.* also found that adequate Vacreator pasteurization, practised in some 200 plants in the United States and Canada (119), was assured by a first-chamber temperature of 191·5° F., and England (120) has outlined the advantages, and demonstrated the efficiency, of Vacreator pasteurization over batch pasteurization. Even where pasteurized milk and cream are being used in the manufacture of ice-cream it is essential that the mix be heat-treated if a product of low bacterial content is to be obtained, and Schmidt (121) and Bartsch (122) have listed the chief sources of contamination; in Shitskova's (123) experiments pasteurization for 5–30 min. at 65–85° C. reduced bacterial numbers by 98·1–99·9%. According to Burbianka (124) ice-cream made from fruit juices has a considerably lower bacterial count than that made from milk, but it is not stated whether this is due to acidity or lower original bacterial content, or both. For the detection of coliform organisms in ice-cream Borneff (125) uses a nitrate-reduction test and the selective action of brilliant green, but in America a solid medium containing sodium desoxycholate is preferred to a liquid one with brilliant green (126). Attimonelli's (127) nitrate-reduction test has been used extensively by Scotti (128) who obtained good agreement between her results with it and with total bacterial and coliform counts; the test is recommended for the routine hygienic control of ice-cream.

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